Cyclical Parthenogenesis and the Evolution of Sex: The Causes and Consequences of Facultative Sex

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Dissertation

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—Why all this silly rigmarole of sex? Why this gavotte of chromosomes? Why all these useless males, this striving and wasteful bloodshed, these grotesque horns, colours, . . . and why, in the end, novels like Cancer Ward, about love?

—W.D. Hamilton, 1975

Ask any person on the street
Why do males and females meet?

Why sex exists, everyone seems to know
It's only evolutionary biologists whose minds are blown

Sex is so complicated and bears so many cost
It seems that over short time this trait should be lost!

Still, reaching from fungi, plants, insects to e.g. a Finn,
Most eukaryotes engage in some form of the original sin

But obligate sex is not the most elaborate solution
Cyclical parthenogens are the masterpiece of evolution

Instead of in all generations looking for a suitable mate
Most of the time they produce clones at a much higher rate

Asexual growth is all nice and fast
But without sex, the population will soon belong to past

Only ephippia can survive freezing or droughts
Thus at some point sex is needed without any doubts!

For this, before winter arrives and everything freezes
Males need to be produced parthenogenetically – just like Jesus

The males need time to mature before donating their sperm
To fertilize ephippia and ensure survival in the long-term

But why is sex linked to such an important trait?
Asexually produced ephippia, this would be great!

And why does something like obligate sex even exist?
When with this, fast clonal growth is completely missed!

And once facultative sex evolved
There are additional problems that need to be solved:

When should they produce males? And when should they mate?
For little daphnia these decisions are crucial for the population's fate

Unravelling these mysteries was yet to be done
And with this, three years ago my PhD has begun…

SUMMARY

Most theories on the evolution of sex consider obligate asexual versus obligate sexual reproduction. The presence of occasional sex, such as facultative or cyclic parthenogenesis, is underappreciated, even though facultative sex seems to combine the best of both worlds: While the cost of sex can be avoided in most generations, almost all of its benefits are gained through occasional sex. To investigate the causes and consequences of such a life-cycle, my thesis combines sex allocation theory in facultative sexual organisms with bet-hedging theory in a collection of several theoretical and empirical studies.

Once facultative sex evolved, there should be strong selection on the timing of sex, and the consequences of this life-cycle offer interesting insights in sex allocation theory and in the conditions when sex is favoured over asexual reproduction. Using *Daphnia* as a model system, I showed that the optimal strategy for *Daphnia* females is to spread the sexual production of dormant eggs across several reproductive events to hedge their bets in unpredictable environments. Furthermore, they should invest in sexual reproduction at high population densities. I could also demonstarte, that natural *Daphnia* populations indeed invest in sexual reproduction when population densities are high and returns of asexual reproduction relatively low. Thus, they invest in sexual reproduction not only in anticipation of upcoming harsh conditions, but also when the relative costs of sex are reduced. Furthermore, I showed that consistent with classical sex allocation theory, *Daphnia* adjust their investment in sons not only to the population density, but also to the current adult sex ratio in the population, which affects the reproductive value of sons and varies within a season.

A link between sex and an ecological function (e.g. dispersal or dormancy) provides a mechanism which can stabilize sex against the invasion of purely asexual lineages: If sex is necessary to produce dispersing or dormant stages, and these are crucial for the survival of a lineage, sex is inevitably stabilized. Here, I showed, that this link can evolve due to condition-dependent investment in risk-spreading strategies. With these results, I provide explanations for the discrepancy between different theoretical predictions on co-varying risk-spreading traits. Lastly, I provide explanations for the evolutionary stability of sexual reproduction and demonstrate that sexual conflict can act as a mechanism to stabilize sex in dense populations, and thus prevent facultative sexual lineages to invade obligate sexual lineages. With this, I provide potential explanations for the true paradox of sex – why obligate sex evolved in the first place – as well as for patterns of geographic parthenogenesis.

Altogether, through a combination of empirical and theoretical methods, I could shed light on conditions that stabilize sex and explain empirical patterns with theoretical models. By considering bet-hedging theory, I provide new insights into sex allocation theory. The *Daphnia* system allowed to test such theoretical predictions, and with this, to connect theoretical models to empirical reality. Furthermore, by incorporating different levels of adaptedness into classical bet-hedging theory, I provide explanations for the co-evolution of risk spreading strategies. Thus, this thesis shows that combining different methods, as well as combining concepts from different, rather isolated fields, can bring a new perspective to longstanding theory.

ZUSAMMENFASSUNG

Die meisten Theorien zur Evolution von sexueller Fortpflanzung vergleichen sexuelle und asexuelle Reproduktion in ihrer obligaten Form. Fakultative sexuelle Fortpflanzung, wie es in zyklischer Parthenogenese (Heterogonie) vorkommt, wird kaum berücksichtigt, obwohl dieser Generationswechsel die Vorteile von ungeschlechtlicher mit geschlechtlicher Fortpflanzung kombiniert: Die Nachteile der geschlechtlichen Fortpflanzung können über die meisten Generationen vermieden werden, während deren Vorteile fast vollständig erhalten bleiben. Um sowohl Konsequenzen als auch mögliche Gründe für die Evolution von fakultativer sexueller Fortpflanzung zu untersuchen, kombiniere ich in meiner Dissertation klassische Theorie zur Geschlechterverteilung mit "Bet-hedging"-Theorie und wende verschiedene theoretische und empirische Methoden an.

Fakultativer Sex bietet die Möglichkeit, Bedingungen zu identifizieren, in welchen sexuelle Fortpflanzung bevorzugt wird. Mit Hilfe von Daphnien als Versuchsorganismus konnte ich zeigen, dass sich optimal verhaltende Daphnien-Weibchen sexuelle Fortpflanzung über mehrere Brutzyklen verteilen, wenn Veränderungen in der Umwelt schwer vorhersehbar sind. Zudem sollten sie ihre Bereitschaft zu sexueller Fortpflanzung erhöhen, wenn Populationsdichten hoch sind. Ausserdem konnte ich zeigen, dass Daphnien in natürlichen Populationen sich tatsächlich eher sexuell Fortpflanzen, wenn Populationsdichten hoch sind und asexuelle Fortpflanzung gleichzeitig ineffizient ist. Dies bedeutet, dass Daphnien den Zeitpunkt von sexueller Fortpflanzung nicht nur an herannahende schlechte Umweltbedingungen ausrichten, sondern auch an Bedingungen, bei denen die Kosten von sexueller Fortpflanzung reduziert sind. Des Weiteren zeige ich in meiner Dissertation, dass *Daphnien* das Geschlechterverhältnis ihrer Nachkommen nicht nur an die Populationsdichte, sondern auch an das Geschlechterverhältnis in der Population anpassen, da dieses den „Reproduktionswert" der Geschlechter beeinflusst.

Sexuelle Fortpflanzung kann evolutionär stabilisiert werden durch eine Verknüpfung von sexueller Fortpflanzung mit Strukturen, die zum Beispiel zur Verbreitung oder zur Überdauerung nötig sind. Dadurch wird verhindert, dass komplett asexuelle Populationen – welche diese Strukturen nicht bilden können – sich uneingeschränkt verbreiten und sexuelle Populationen verdrängen können. Ich zeige, dass so eine Verknüpfung entstehen kann, wenn Individuen sowohl mehr in sexuelle Vermehrung als auch in Überdauerungs- und Verbreitungsstrukturen investieren, wenn sie schlecht an die gegebenen Umweltbedingungen angepasst sind. Als letztes zeige ich, dass in Populationen mit hoher Dichte ein „Geschlechterkonflikt" verhindern kann, dass fakultativ sexuelle Abstammungslinien sich gegen obligat sexuelle durchsetzten. Damit biete ich eine mögliche Erklärung zum „wahren Paradoxon der sexuellen Fortpflanzung": warum obligate sexuelle Fortpflanzung überhaupt evolvieren konnte.

Durch eine Kombination von empirischen und theoretischen Herangehensweisen konnte ich Bedingungen identifizieren, welche sexuelle Fortpflanzung evolutionär stabilisieren. Ausserdem biete ich neue Erkenntnisse zu Theorien der Geschlechterverteilung und konnte theoretische Prognosen in *Daphnien* Populationen testen. Insgesamt verbindet diese Dissertation verschiedene Methoden und zeigt, dass die Integration von Theorien aus verschiedenen Fachbereichen neue Perspektiven auf langjährige Enigmen bringen kann.

YHTEENVETO

Useimmat seksin evoluutiota koskevat teoriat vertailevat ehdotonta aseksuaalista lisääntymistä ehdottomaan seksuaaliseen lisääntymiseen. Aseksuaalisen lisääntymisen täydentäminen seksillä, kuten ehdollinen tai syklinen partenogeneesi, on aliarvostettua, vaikka se näyttäisi yhdistävän molempien edut: satunnainen seksuaalinen lisääntyminen auttaa välttämään seksin kustannukset useimmissa sukupolvissa, silti säilyttäen lähes kaikki seksin edut. Väitöskirjassani tutkin tällaisen elinkierron syitä ja seurauksia yhdistämällä teoriat ehdollisesti seksuaalisten organismien sukupuolijakaumista sekä riskien jakamisesta.Ehdollisesti seksuaaleissa systeemeissä voimme myös tutkia ehtoja joiden vallitessa seksuaalista lisääntymistä suositaan aseksuaalisen sijaan, sekä ehtoja milloin ehdollinen lisääntyminen on puhtaita strategioita kannattavampaa. Ehdollinen seksi tarjoaa mielenkiintoisen systeemin missä tutkia sukupuolijakaumia, sekä etsiä ehtoja jotka suosivat seksuaalista lisääntymistä.

Daphniaa mallilajina käyttäen näytän, että *Daphnia* naaraiden tulisi jakaa horrostilassa olevien munien seksuaalisen tuotannon useisiin eri lisääntymiskertoihin vähentääkseen niihin kohdistuvia riskejä ennalta-arvaamattomissa ympäristöissä.

Tämän lisäksi osoitan, että *Daphnia* naaraiden tulisi lisääntyä seksuaalisesti tiheissä populaatioissa, kuten havaitsin tapahtuvan luonnossa. Ne siis panostavat seksuaaliseen lisääntymiseen kun seksin suhteellinen kustannus on pieni, huonoihin olosuhteisiin valmistautumisen lisäksi. Lisäksi huomasin, kuten klassinen sukupuolijakaumien teoria ennustaa, että Daphnia naaraat muokkaavat poikatuotantoaan sekä populaatiotiheyden että sen hetkisen populaation sukupuolijakauman mukaan. Poikien osuus populaatiossa vaihtelee kausittain vaikuttaen niiden arvoon jälkeläisinä. Väitöskirjassani tarjoan myös selityksen sille, miksi ehdoton seksuaalinen lisääntyminen voi pysyä suojattuna ehdollista seksuaalista lisääntymistä vastaan: sukupuolten välinen konflikti toimii mekanismina joka stabiloi seksin tiheissä populaatioissa. Sama mekanismi tarjoaa selityksen partenogeneesin maantieteelliselle levinneisyydelle ja ehdollisen seksin harvinaisuudelle.

Toisaalta mekanismi joka suojaa seksuaalisen lisääntymisen aseksuaalisia linjoja vastaan on seksin suhde ekologisiin funktioihin (esim. levittäytyminen tai horrostautuminen): jos lajin elintilaa laajentavat tai horrostavat yksilöt syntyvät vain seksin kautta, ja nämä ovat lajin säilymiselle vaadittavia ominaisuuksia, seksi on väistämättä stabiloitu. Väitöskirjassani näytän, että tämä suhde voi syntyä kunto-riippuvaisesta sijoittamisesta strategioihin jotka jakavat riskejä. Tulokseni tarjoavat selityksen ristiriidoille ennusteiden välillä joita eri riskien jakamisten teoriat ovat tuottaneet.

Yhdistämällä empirian ja teorian pystyin selventämään ehtoja jotka stabiloivat seksin ja jotka selittävät aiemman empiirisen ja teoreettisen tutkimuksen välisen suhteen. Riskien jakamisen teorian avulla pystyin tuomaan uutta ymmärrystä sukupuolijakaumien teoriaan. *Daphnia* tarjosi mahdollisuuden tutkia tuottamiani teoreettisia ennustuksia, ja siten yhdistämään teoreettiset mallit empiiriseen todellisuuteen. Lisäämällä eri sopeutuvuuksien tasot klassiseen riskien jakamisen teoriaan löydän selityksen eri riskien jakamis-strategioiden koevoluutiolle. Kokonaisuutena väitöskirjani osoittaa, että verrattain eristäytyneiden alojen metodien ja käsitteiden yhdistäminen voi tuoda uusia näkökulmia pitkäaikaseen teoriaan.

CHAPTER I

General Introduction

Reproduction is a key element of the concept of biological fitness and thus is a main driver of life histories of all living organisms. Genes that increase reproductive success have been shaped by natural selection. However, sexual reproduction with internal fertilisation, as performed by mammals, is by far not the only way of reproduction and not the only form of sex (Aanen et al. 2016) The broadest definition of sex is the combination of genes from different individuals (Lehtonen and Kokko 2014). Following this definition, sex is often associated with reproduction, but it is actually not a reproductive process itself, and sex can also occur without reproduction, as e.g. conjugation (DNA sharing) in bacteria. However, in this thesis, I will focus on eukaryotes and I will use the term sex equivalent to sexual reproduction, in the sense of combination of gametes from different individuals, and I will mostly ignore sex isolated from reproduction.

Regular meiotic sex evolved at the origin of eukaryotes or soon thereafter (Cavalier-smith 2002). Ever since, meiosis is the most common mechanism for the separation of homologous genomes and the formation of haploid gametes, which then fuse during fertilization, and this mechanism is basically identical in most organisms (John 1990, Ramesh et al. 2005). However, while the basic structure of meiosis is well preserved, there is huge diversity in the form of sexual cycles among eukaryotes. In humans, for example, the diploid stage takes several decades, while the haploid stage only lasts hours, whereas e.g. mosses spend most of their life-cycle in the haploid stage (Bengtsson 2009) and in e.g. aphids, between two meiotic cycles are cycles of asexual reproduction (Simon et al. 2002). Sexually reproducing organisms also differ in their appearance of the haploid stage: In anisogamous organisms, a clear differentiation of many small gametes (male gamete) and large, nutrient providing gametes (female gamete) evolved, whereas in isogamous species, there is no such differentiation. In hermaphrodites (organisms where the same individual can produce male and female gametes), various forms of the life-cycle evolved, from simultaneous hermaphroditism, where one individual produces both gametes and self-fertilization can occur, to sequential hermaphroditism, where individuals change their sex during their lifetime. These are just a few examples of the intriguing diversity in sexual cycles that are found in nature.

Like sexual reproduction, asexual reproduction can take many forms. Asexually produced offspring are generally produced without any fusion of gametes (with some special cases of automixis), and offspring inherit the genetic material only from their mother (or in rare cases of androgenesis, only from their fathers (Schwander and Oldroyd 2016)). What asexual organisms (according to this definition) have in common, is that they dropped out of the regular meiotic cycle that sexual organisms engage in. Some organisms can produce offspring asexually through unspecialized cells, such as budding or vegetative growth. Other asexually reproducing organisms reproduce via parthenogenesis (greek for “virgin creation”), where unfertilized eggs develop successfully into offspring. Parthenogenesis occurs in many plants (where parthenogenesis is a process of apomixis), invertebrates (e.g. aphids, rotifers, *Daphnia*, stick insects, mayflies, some ants and bees and parasitic wasps), and vertebrates (e.g. reptiles, amphibians, birds, sharks (see Lampert 2008)). Organisms can express

different levels of parthenogenesis, where it can either be obligate, facultative (see: The best of both worlds: facultative sex) or accidental. Additionally, parthenogenetically produced offspring can either be female (thelytoky) or male (arrhenotoky, e.g. in haplodiploids,). In this thesis, I usually refer to thelytoky when speaking of asexual reproduction, and I use facultative sex and facultative parthenogenesis interchangeably.

Compared to asexual reproduction, sexual reproduction seems very complicated. However, sexual reproduction is prevalent in most animal taxa and most eukaryotes engage in at least some form of sex. There are only few exceptions that do not invest in sex at all: only ~0.1% of named animal species are considered exclusive asexuals (Vrijenhoek 1998). Among plants, still less than 1% of angiosperm species are considered substantially asexual (Asker and Jerling 1992, Otto 2009) even though many plant species are capable of vegetative reproduction (via rhizomes, runners, tubers, bulbils, etc.) and/or the production of asexual seeds via apomixis. In contrast, the majority of protists and fungi can reproduce asexually by fissioning, budding, or spore production. Still, the prevalence of sexual reproduction is a key question in evolutionary biology. To understand why the prevalence of sex is considered the "Queen of problems" in evolutionary biology (Bell 1982), we need to understand the costs and benefits of different reproductive modes.

The costs of sex

To determine the costs of sex is not as straightforward as it might seem at first sight. It is important to note that here, I am not really interested in absolute costs of sex, but rather in the cost of sex compared to other reproductive modes, for example asexual reproduction. These costs may differ, depending on the exact system (and definition of sex), as all other costs than physiological costs of meiosis are not costs of sex per se, but arise from the way sex is performed.

As mentioned above, there are physiological costs of meiosis itself. Meiosis takes much longer than mitosis in unicellular organisms (Otto 2009, Lehtonen et al. 2012). These are basically opportunity costs: A yeast cell, for example, could go through mitosis 8 times during the time that is needed for conjugation and reorganization. In the yeast case, this results in a 256-fold cost of sex (Burt 2000, Beukeboom and Perrin 2014).

Additionally, the building of reproductive organs (e.g. gametes) or secondary sexual traits can incur physiological costs. Once the required structures are built, there can be additional costs: To be able to reproduce, a sexual organism must find a suitable mate (or its gametes). The cost of finding a mate can be particularly high at low population densities. Then, once a suitable mate has been found, mating facilitates the spread of sexually transmitted diseases (Lockhart et al. 1996, Otto 2009) and might enhance predation risk, especially when morphological and behavioural displays are involved. Furthermore, the mating process itself is not only energetically costly, but can also entail opportunity costs – mating can take time in which no other activities (e.g. feeding) are possible.

In anisogamous systems, the so-called twofold cost of males emerge because males typically do not transmit material resources into offspring, and basically only contribute genetically to the next generation (Maynard-Smith 1978). Thus, a sexual lineage that produces males suffers from the twofold cost of producing males. Imagine a sexual lineage, where a female invests half of her resources in the production of males (which do not provide any resources to

the offspring). Compared to this sexual lineage, the asexual lineage that only produces daughters is twice as efficient in converting resources into offspring because males do not produce offspring themselves (Lehtonen al. 2012). In addition to these twofold costs, males can also have additional direct negative effects on female fitness through sexual conflict (Rankin and Kokko 2007). Such negative effect can arise through sexual harassment. Males of many species harass females to achieve mating (Rowe et al. 1994), whereas females trying to resist such harassment suffer fecundity or survival costs in a wide range of systems (Shine et al. 2000, Rönn et al. 2006, Gosden and Svensson 2009).

Measuring the costs of sex in nature is rather complicated. First, one needs to think about what the sexual lineage should be compared to. Then, the timescale is crucial as well – should one consider short-term net benefits or should one consider sex in the long-term (Lehtonen et al. 2012)? Despite these challenges in measuring the costs of sex, several empirical studies have investigated how sex affects different fitness components (see Lehtonen et al. 2012). Some studies find that asexuals have higher mortality or lower fecundity (e.g. Roth 1974, Kearney and Shine 2005), whereas other studies find no difference or a fitness reduction of sexuals up to a two-fold costs of sex (Gibson et al. 2017). Thus, determining the costs of sex in a natural system is not as straightforward as theory predicts and these costs are not always twofold, but rather depend on the particulars of the sexual system and to what alternative sex is compared.

The benefits of sex

While asexual reproduction is often advantageous in the short term, mainly because of demographic benefits (see above), the benefits of sexual reproduction are rather of genetic nature arising from recombination, and pay off in the long term. There are immediate effects of recombination on fitness, including DNA reparation and transmission of selfish genes (Goddard et al. 2001). However, these direct benefits have problems explaining the evolution and maintenance of sex (Rice and Friberg 2009, Beukeboom and Perrin 2014) and I will focus on indirect benefits of recombination, which are based on the consequences through mixing genetic material of two individuals.

The advantage of sex and recombination can be summarized as increased efficiency of natural selection in the presence of recombination. The underlying concept is that by combining genetic material from different backgrounds, sex and recombination lead to genotypes that are better adapted, and consequently form populations that can better adapt to environmental conditions (Hartfield and Keightley 2012). Williams (1975) summarized this idea by comparing different mating strategies in a fluctuating environment to buying lottery tickets: The asexual strategy is represented by a strategy that buys lots of tickets with the same number, whereas sexual reproduction matches a strategy that buys fewer tickets, but containing different numbers. Thus, sexual lineages are more likely to persist in the long term, especially in changing environments.

One benefit arising from sex is breaking apart interfering mutations, and thereby increasing the response to selection. This can happen by preventing background-trapping of mutations. Background-trapping occurs when two beneficial mutations arise at separate, linked loci in a population. In an asexual population, these two mutations are trapped in their background and to be combined in one genome, both mutations have to sequentially arise in the same individual. In sexual populations, two beneficial mutations can be combined through recom-

bination, quickly generating a fitter individual (Fisher 1930, Muller 1932). Similarly, if beneficial mutations arise in a low fitness genetic background, they are trapped in a low fitness background and get purged in asexual populations ("Ruby in the rubbish process", Fisher 1930, (Charlesworth et al. 1992,Peck 1994), whereas mutations can escape the low fitness background in sexual populations and increase the chances of getting fixed (Charlesworth et al. 1992). Along the same line, a beneficial mutation that fixes in an asexual population drags mildly deleterious or neutral mutations with it, leading to progressive evolution at one locus combined with retrogressive evolution at another ("hitchhiking decay", Rice 1987).

Besides background-trapping, other mechanisms can slow down progressive evolution and increase retrogressive evolution. If two beneficial mutations arise in two clonal lineages, the rate of accumulation of these mutations will be slowed down, due to competition of the clonal lineages ("clonal interference", Clarke et al. 1994). On the other hand, the genomes of asexual populations irreversibly accumulate deleterious mutations (Muller 1964). This process is also described as Muller's ratchet, where with every click of the ratchet, individuals with the least mutated genome are lost due to drift, leading to an increased mutation load in asexual populations over time. However, a small amount of recombination is sufficient to stop the ratchet (Charlesworth et al. 1992).

The increased efficiency of selection in sexual populations builds the genetic foundation for the benefits of sex due to "Red Queen" dynamics (Van Valen 1973, Maynard-Smith 1978, Hamilton 1980, Bell 1982). The underlying concept of a Red Queen process is negative frequency-dependent selection through higher fitness of rare host genotypes in host-parasite interactions. If parasites preferentially infect common host genotypes (due to local adaptation to these hosts), sexual reproduction and recombination produce new host genotypes, which are less susceptible and thus enjoy higher fitness (for details and empirical tests of this hypothesis, see e.g. Neiman et al. 2009).

Ecological conditions favouring sex and geographic patterns

Theoretical work has identified several ecological conditions favouring the maintenance of sexual reproduction. These conditions include changing selection over time (e.g. Red Queen dynamics) and space (e.g. tangled bank hypothesis (Bell 1982, Scheu and Drossel 2007, Otto 2009)) and the unpredictability of such changes. Sex rapidly creates variable offspring genotypes, which increases the chances that at least some of the offspring are adapted to the changing conditions. If these changes are unpredictable, sex can act as a strategy (Li et al. *in press*). Additionally, constraints such as genetic imprinting in mammals can constrain the evolution of occasional sex, as it prevents asexual reproduction (Engelstädter 2008). In facultative sexual species, sex is commonly constrained by an association of sexual reproduction with an ecological function – e.g. dispersal or dormancy – which can stabilize sex (Stelzer and Lehtonen 2016). If harsh conditions such as winter can only be survived via resting eggs, and such can be only produced sexually, sex can be 'incidentally maintained'. However, the intriguing question that arises is why such an association evolved in the first place.

In nature, geographic distributions of sexually and asexually reproducing species can identify conditions favouring sex. Asexually reproducing taxa and their sexual relatives often inhabit distinct ranges (see Tilquin and Kokko (2016) for a recent review). Mostly, three patterns are described, where asexuals have a wider distribution (Vrijenhoek and Parker 2009), occur at higher latitudes (Bierzychudek 1985) and/or at higher altitudes (Kearney 2005).

These patterns suggest that asexual reproduction may be adaptive in newly colonized and more marginal environments. In these environments, populations are often small and have low genetic variation, and sexual individuals might have difficulties in finding a mate ("mate assurance hypothesis" (Baker and Baker 1965, Hörandl 2009). Thus, females that are able to reproduce without fertilization by a mate might have a fitness advantage. Furthermore, in newly colonized environments specialized predators, pathogens and competitors might be absent, reducing selection pressures that are thought to favour sexual reproduction (Glesener and Tilman 1978, Hamilton 1980, Lively and Jokela 2002).

These well-documented patterns of geographic parthenogenesis seem to contrast to accumulating evidence, that in general sex seems to be associated with stressful conditions (see Ram and Hadany (2016) for a recent review), where increased adaptive evolution is especially beneficial. However, for a sexual lineage to persist in the presence of asexual competitors there needs to be either a much less than two-fold cost of sexual reproduction, which prolongs the time available for a sexual lineage to benefit from long-term effects, or some sort of spatial refuge preventing asexuals from outcompeting sexual lineages over their entire range in the short term (Rice and Friberg 2009).

The best of both worlds: facultative sex

Among eukaryotes, sex dominates as the reproductive system, leading to the queen of problems in evolutionary biology: The paradox of sex (Bell 1982). Given the many cost of sex (outlined above), why has sex become so successful and is prevalent in most eukaryotes? However, the actually more intriguing question is: Why is obligate sex maintained, and conversely, why is facultative sex so rare (Burke and Bonduriansky 2017)? Facultative sexual organisms can alternate between sexual and asexual reproduction, and such occasional sex is thought to provide most of the benefits of sex, while avoiding most of its cost (Hurst and Peck 1996, D'Souza and Michiels 2010, but see Peck and Waxman (2000)). Asexual reproduction can allow for fast population growth during most generations, while occasional sex allows for sufficient recombination to avoid the genetic costs of asexuality (Green and Noakes 1995, Peck and Waxman 2000, reviewed in D'Souza and Michiels 2010).

Most theories on the evolution of sex consider obligate asexual and obligate sexual reproduction (Hurst and Peck 1996), whereas facultative sex in predominantly asexual organisms is underappreciated (Som and Reyer 2007). Such occasional sex prevents the accumulation of deleterious alleles (Charlesworth et al. 1993), promotes the fixation of beneficial mutations (Green and Noakes 1995, Peck 1994), facilitates adaptation (Yamauchi 1999) and enhances evolvability (Lynch and Gabriel 1983) as efficiently as obligate sex does. Also, under negative frequency-dependent selection (the assumption of the Red Queen hypothesis), facultative sexual organisms can invade populations with obligate sex or obligate asexual reproduction (Yamauchi 1999).

When estimating the cost of facultative sex, it matters whether the costs of sex are calculated per cycle (can include several asexual/sexual generations) or averaged per generation (Rispe and Pierre 1998). If the costs are averaged over generations, the cost of facultative sex is two-fold as an asexual mutant can double its frequency compared with that of a facultative sexual withinn one complete generation of sex (D'Souza and Michiels 2010, Rispe and Pierre 1998). If calculated per cycle, the costs of sex are dependent on the frequency with which sex is engaged, and costs are strongly reduced in species with only rare sexual generations

(Charlesworth 1980). A reduced cost of sex in systems with occasional sex is widely accepted in the literature (e.g. Lewis 1987, Green and Noakes 1995, Hurst and Peck 1996, see D'Souza and Michiels (2010) for a review). Therefore, species with occasional sex gain the benefits of sexual reproduction without paying much of its costs, at least theoretically. Furthermore, when adapting to new environments – which represents a condition that facilitates sexual reproduction – condition-dependent (and thus facultative) sex can be favoured over obligate sex (Hadany and Otto 2009). The problem then posed is why such a beneficial strategy is apparently rather rare in nature (Burke and Bonduriansky 2017), respectively why obligate sex evolved at all. If facultative sex is as advantageous as (or even more advantageous than) obligate sex, to explain its rarity it must either be evolutionarily unstable, or else potentially many organisms are yet to be identified as facultative sexual (D'Souza and Michiels 2010).

Identifying facultative sexual species in nature is challenging and there is not much information available about the exact number of taxa engaging in a life-cycle that combines sexual and asexual reproduction (D'Souza and Michiels 2010). Verifying occasional sexually produced generations in otherwise parthenogenetic species is challenging because they might be rare (Schurko et al. 2009) or cryptic and occur unpredictably or only occur in response to certain environmental stimuli (Dacks and Roger 1999). Additionally, detecting cases of rare parthenogenetic events in usually sexual species or the occurrence of sexual events in usually parthenogenetic species are difficult to verify. By simply looking at population genetic structure of a population, it is difficult to estimate the frequency of sex, because the signatures of facultative sex are similar to the genetic signatures of obligate sex (Green and Noakes 1995).

So far, there is evidence that the majority of protists are facultative sexual (Dacks and Roger 1999) and there is increasing evidence for facultative sexual metazoans. Most sexually reproducing plants are capable of vegetative reproduction, and most apomictic plants (~ parthenogenetically reproducing plants) engage at least to some degree in sex (D'Souza and Michiels 2010, Asker and Jerling 1992, Richards 2003). Also, most parthenogenetic animals engage in some form of occasional sex (e.g. Goddard and Schultz 1993, Schurko et al. 2009). Many invertebrates engage in cyclical parthenogenesis, e.g. rotifers (Barnes 1982), aphids (Simon et al. 2002), *Daphnia* (Ebert 2005, Schön et al. 2009). Even many vertebrates, which usually reproduce sexually, can occasionally (albeit probably accidentally) produce offspring via parthenogenesis, e.g. snakes , lizards, sharks and birds such as zebra finches or chickens (see Lampert 2008). Thus, there seems to be no constraint that prevents facultative sex (such as e.g. genetic imprinting in mammals) in many taxonomic groups.

Consequences of facultative sex

In facultative sexual organisms, there should be strong selection on the timing of sexual reproduction (Snell 1987, Serra and Carmona 1993). Asexual reproduction offers higher population growth, as the twofold cost of producing males can be avoided (Maynard-Smith 1978, Lehtonen et al. 2012). Sex offers the benefits outlined above, and in many cases is linked to the production of resting or dispersing stages. In many facultative sexuals, a female cannot reproduce both sexually and asexually in the same reproductive event, the two reproductive modes are traded off against each other, influencing the optimal timing of the induction of sexual reproduction (Snell 1987, Serra and Carmona 1993, Serra and Snell 2009). An association of sexual reproduction with an ecological function (e.g. dispersal or dormancy), which

is necessary to survive harsh conditions (e.g. winter), leads to additional complexity in the selection on the timing of sex. Sexually produced resting stages need to be produced before the onset of inhospitable periods. If sexual reproduction is induced late in the period of favourable environmental conditions (the "growing season"), the lineage undergoes a long period of asexual growth and is highly competitive within a season. However, if there is uncertainty about the onset of inhospitable periods, and the season ends before sex is induced, the whole lineage goes extinct. On the other hand, if sex is induced early in the growing season, the lineage loses competitive potential due to demographic costs of sex.

The fact that sex needs the presence of mature males can add to the complexity of sex allocation decisions in some cyclical parthenogens such as *Daphnia* (see below). Switching to sexual reproduction as a female is pointless when no mature males are around that could fertilise the eggs. Similarly, producing males is pointless if in the near future no females produce sexual eggs. Thus, sex allocation decisions (what sex to produce) are not independent from decisions on the reproductive modes.

Concerning the production of males, sex allocation theory predicts that if fitness returns of daughters are different from the returns of sons, selection will favour an increasing investment towards the sex with the higher reproductive value (West and Sheldon 2002, West et al. 2002). Therefore, the offspring sex ratios should become biased towards the sex with the currently higher fitness returns. When sex is facultative, there is usually strong variation in the population sex ratio over the season, leading to variation in the returns of producing sons or daughters. This can potentially lead to selection on adjustment of offspring sex ratio, depending on the sex ratio of the current population. Thus, exhibiting a facultative sexual life-cycle has several consequences and strongly influences selective pressures on the timing of sexual reproduction and sex allocation.

Facultative sex and bet-hedging theory

The combination of unpredictable environments with the trade-off structure of sexual vs. asexual reproduction offers an interesting framework for bet-hedging theory, where mean fitness is traded off against a reduction in variance of fitness (Starrfelt and Kokko 2012). The classical example of bet-hedging provides explanation on why the germination rate of plant seeds can evolve to be less than one (i.e., variable seed dormancy length). Consider two lineages of an annual plant, one where all seeds germinate the year following their production and another lineage where seeds can delay germination (the 'bet hedger'). In a year with good environmental conditions, the lineage with complete germination (germination rate = 1) will have a higher fitness, as more seedlings hatch and reproduce. However, in a bad year (e.g. drought, freezing, flood), where germinated seedlings cannot survive, the bet hedger only loses a fraction of its seeds and leaves seeds to germinate in future years, and thus has a fitness advantage over the lineage with complete germination, which loses all seedlings. Bet-hedging reduces the variance in fitness over several events, but also reduces arithmetic mean fitness, as bet-hedging strategies are costly, because e.g. seeds in a seed bank are exposed to higher predation risk. Dormancy (Spencer et al. 2001), dispersal (Vitalis et al. 2013), multiple mating (Yasui 2001), nutrient storage in rhizomes (Ratcliff & Denison 2010) and sex (Li et al. *in press*) have also been shown to represent bet-hedging strategies. In this thesis, I will investigate the consequences for sex allocation when the onset of dormancy, rather than its termination, is considered as a bet-hedging trait. I will also investigate the co-

evolution of multiple bet-hedging traits when allowing for condition-dependent investment in risk-spreading strategies.

Objectives

In this thesis, I study an underappreciated way to deal with temporally and spatially varying environments: facultative sex. I aim to investigate the causes and consequences of this life-cycle and relate these findings to the evolution and maintenance of sex. To do so, I applied a combination of theoretical and empirical approaches to be able to provide general patterns and predictions from theoretical models, which that grounded and evaluated in empirical reality.

The thesis is inspired by the *Daphnia* life-cycle, which combines sexual and asexual reproduction within a season. I aim to find the optimal timing of male production and induction of sexual reproduction using a genetic algorithm. Furthermore, I aim to identify conditions when it is beneficial to invest in male production and sexual reproduction, by observing population dynamics in nature. I also aim to investigate sex allocation with respect to the current population sex ratio to test predictions of classical theories in a system where sex is facultative. Therefore, I perform mesocosm experiments, where I can easily manipulate the population structure. Furthermore, I use individual-based simulations to identify patterns of the co-evolution of sex, dispersal and dormancy – a link that is widespread in nature. This link can prevent an invasion by asexual lineages and thus stabilise sex and facultative sex. By investigating this link, I aim to provide an explanation for the discrepancy of theoretical predictions and its occurrence in natural systems. Lastly, I aim to integrate the effect sexual conflict at different population densities on the evolution of sex in facultative sexual organisms. Using deterministic simulations, I can study the interaction of male harassment and female resistance at different population densities. Altogether, I aim to combine different theoretical and empirical approaches and general concepts from different fields to improve understanding of the causes, consequences and implications on the evolution of sex of facultative sex.

Daphnia magna at the southern coast of Finland

Daphnia offer the ideal model system to investigate the consequences of facultative sex empirically. Female *Daphnia* are iteroparous – they have several reproductive events over their lifetime – with overlapping generations within a season. At each reproductive event, females have three options: asexually produce males or females, or sexually produce resting eggs (ephippia), which undergo a dormant period before hatching into females (Figure 1 in Chapter II, electronic Appendix, Figure S1). Parthenogenetically produced clutches have strongly biased sex ratios and contain up to >100 eggs (Hebert 1978), whereas ephippia contain maximally two eggs. The sex of asexually produced clutches is environmentally determined, with daughters as well as sons being genetically identical to their mothers.

At the southern coast of Finland, *Daphnia* live in small freshwater ponds. These habitats are ephemeral, as the ponds freeze in winter and often dry up during warm periods with low precipitation in summer. In laboratory experiments, male production and sexual reproduction are induced in *Daphnia* according to seasonal environmental cues, most notably day length as well as population density (Carvalho et al. 1983, Stross and Hill 1965, Kleiven et al. 1992, Berg et al. 2001).

Daphnia provide an optimal system to connect sex allocation decisions with bet-hedging theory and investigate the consequences of such a complex life-cycle. Studying species with such mixed reproductive modes can enhance our understanding of the evolution and maintenance of sex, because we consider species where the mode of reproduction is not constrained as e.g. in mammals.

Thesis outline

In chapters II-IV, I investigated various consequences of a facultative sexual life-cycle. Such a life-cycle presents a challenge for sex allocation, because females allocate resources not only between male and female offspring, but also between sexual and asexual reproduction. To investigate these questions, I used *Daphnia* as model system. In the cyclical parthenogen *Daphnia,* females can parthenogenetically produce sons or daughters or produce haploid dormant eggs in an ephippium, which need to be fertilized by a male. These sexually produced dormant eggs are the only means to survive harsh conditions. Thus, at every reproductive event, a female has three options: produce males or females asexually, or produce ephippia sexually. Within a reproductive season, the timing of when to produce males and mate with them is critical for fitness because of a trade-off between asexual reproduction, which provides short-term fitness, and sex, which is needed for long-term survival. Especially in environments where the end of the season is unpredictable, bet-hedging in the induction of sexually produced resting eggs might influence sex allocation decisions.

In **chapter II**, I have constructed a model to find the optimal sex allocation strategy in *Daphnia*-like populations when either direct or only indirect cues of the season end are available. Sex generally occurs late in a season but is induced earlier in unpredictable environments, and is even more spread within the season if only indirect cues are available. Furthermore, I could show that sex that coincides with high population densities represents a bet-hedging strategy in the proposed system. In **chapter III**, I followed natural *Daphnia* populations and investigated the population dynamics and the occurrence of sexually reproducing females within a season. I found that sexual reproduction increases with population density and small asexual clutch size, and thus when its relative costs (compared with asexual reproduction) are low. In **chapter IV,** I investigate the allocation in male and female offspring in natural populations and performed mesocosm experiments to disentangle the effect of population density and adult sex ratio on male production. I found that male production increases with population density, but this effect was dampened in male biased populations.

In **chapter V,** I investigated the link between sex and dispersal or dormancy, which is often found in facultative sexual systems. I briefly reviewed the variety of taxa where such a link is found in nature and modelled the expected co-variation patterns of reproductive mode, dispersal and dormancy in the context of local adaptation and spatio-temporally fluctuating environments. I found that while between-species, there exists the predicted negative relationship, positive co-variation can arise within-species due to condition-dependent investment in escape mechanisms. Furthermore, I show that different risk-spreading strategies have dynamic consequences that can feed back on other strategies, and are thus not completely interchangeable.

In **chapter VI,** I investigated the role of male sexual harassment interacting with population density on the maintenance of sex in facultative sexual species. I assume that females can try

to resist the mating attempts of males. However, if resisting is costly, under certain conditions it might be beneficial for a female to accept mating instead of insisting on an asexual life-cycle. Our model shows that resisting male mating attempts pays off at low population densities, leading to the extinction of males and an obligate asexual population. Facultative sex persists at an intermediate density range, and at high densities there is selection against resistance and obligate sex evolves.

Finally, in **chapter VII** I summarized the progress of this PhD on the causes and consequences of facultative sex and discuss the application of the findings in a broader context of the evolution and maintenance of sex.

APPENDIX CHAPTER I

Electronic Appendix: Video from the *Science* Dance your PhD contest:

https://www.youtube.com/watch?v=7LR5m29wmqc

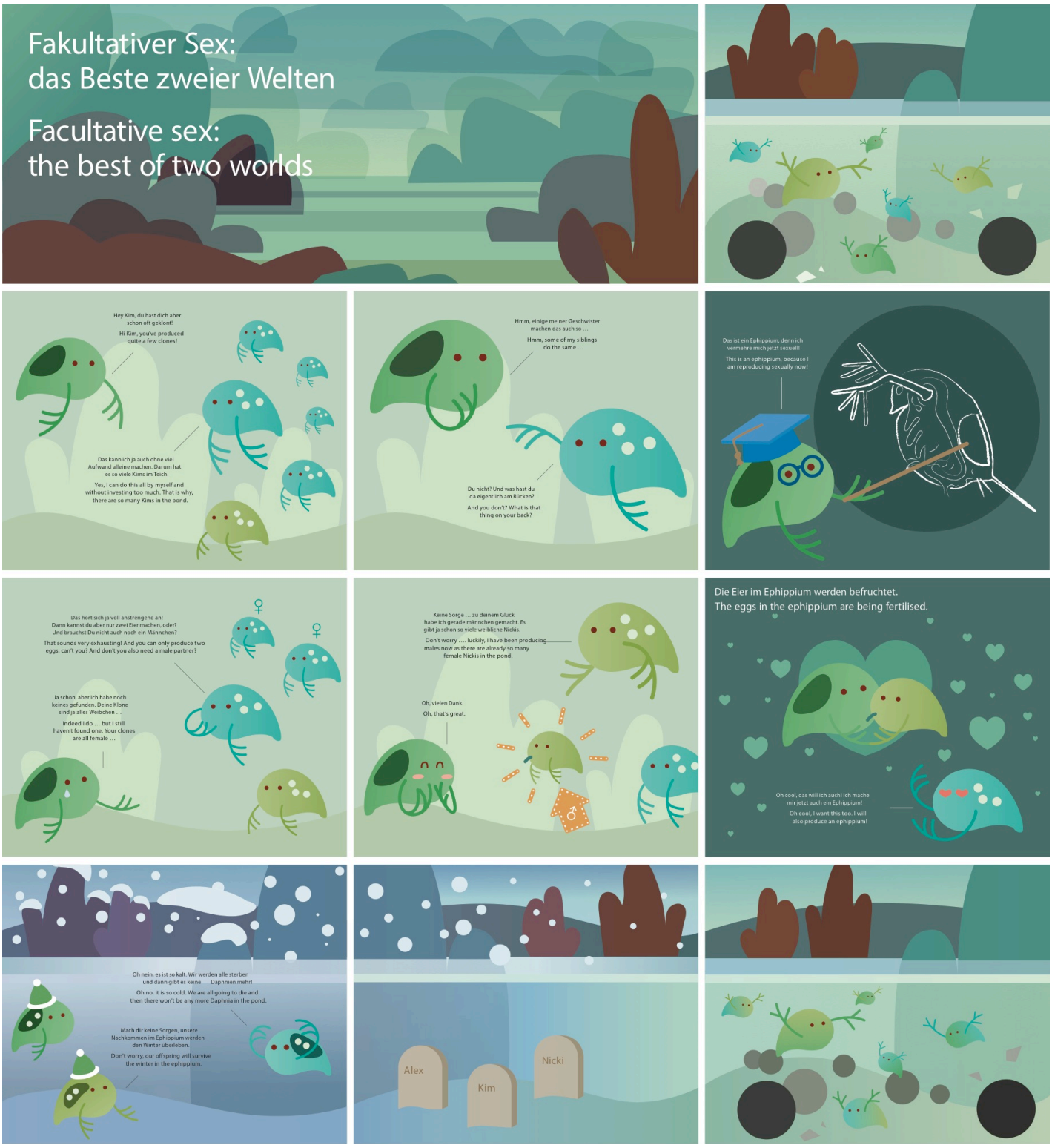

Figure S1 Scientific outreach comic for the Zoological Museum Zurich

CHAPTER II

Sex Allocation Theory for Facultative Sexual Organisms Inhabiting Seasonal Environments: The Importance of Bet-Hedging

Dormancy through sex

Unpredictable season

Bet-hedging pays off

Nina Gerber, Isobel Booksmythe & Hanna Kokko. *In Revison* for *The American Naturalist*.

Abstract

Adaptive explanations for dormancy often invoke bet-hedging, where reduced mean fitness can be adaptive if it associates with reduced fitness variance. Sex allocation theory typically ignores variance effects and focuses on mean fitness. For many cyclical parthenogens, these themes become linked, as only sexually produced eggs undergo dormancy needed to survive harsh conditions. We ask how sex allocation and the timing of sex evolve when this constraint exists in the form of a trade-off between asexual reproduction and sexual production of dormant eggs — the former being crucial for within-season success, the latter for survival across seasons. We show that male production can be temporally separated from or co-occur with sex, depending on whether direct (time) or indirect (population density) cues of the season's end are available, and whether population growth is density-dependent. Sex generally occurs late in the season, but is induced earlier in unpredictable environments. When only indirect cues are available, the temporal spread of sex, and with it the production of dormant stages, is even larger, and given sufficient mortality leads to endogenous population cycles in which frequent sex coincides with high densities. In all scenarios, algorithms maximizing geometric mean fitness have reduced fitness variance compared to a hypothetical non-bet-hedger, confirming that the timing of male production and sex in facultative seasonal settings can be bet-hedging traits.

Introduction

Temporal variation in the environment leads to a major challenge: how do organisms adapt to (often unpredictably) fluctuating environments? If reliable cues are available, plasticity can allow organisms to adjust their phenotype to current environmental conditions (Via and Lande 1985). However, often organisms cannot perfectly predict the demographic consequences of their current phenotypic choices. Finding the best life history solutions can become challenging (McNamara et al. 2016), as simple maximization of mean fitness must be replaced with a more complicated set of trade-offs between mean fitness and its variance within and among individuals, summarized as bet-hedging theory (Slatkin 1974, review: Starrfelt and Kokko 2012).

Dormancy is a classic example of a trait that has been suggested to evolve due to bet-hedging (Evans and Dennehy 2005, Gourbière and Menu 2009). It has attracted much attention in plants (Gremer et al. 2012, Tielbörger et al. 2012, Gremer and Venable 2014) where germination probabilities are predicted to evolve to remain below 1, i.e., not all seeds will germinate in the same season. Recent work considers analogous cases in animals such as rotifers (García-Roger et al. 2014) and crustaceans (Pinceel et al. 2017), where eggs hatch after a variable delay. The general idea is that variable dormancy length can be beneficial if seasons vary in the suitability of conditions for the germinating (hatching) offspring; bet-hedging strategies buffer the risk of reproductive failure as not all offspring develop to experience the same environment (Cohen 1966).

In many organisms that engage in facultative sex (cyclical parthenogens), a mirror image of the plant germination case is encountered: the bet-hedging aspect of dormancy relates to its onset, rather than its termination (though these can also coevolve, Spencer et al. 2001). In cyclical parthenogens, some generations are asexually produced and others sexually, a pattern that has evolved independently in several taxa including *Daphnia* (Decaestecker et al. 2009), various insects (Simon et al. 2002; Burke et al. 2015), rotifers (Aparici et al. 1998) and many plants (Bengtsson and Ceplitis 2000). In such taxa sexual reproduction is often tightly coupled with dormancy and/or dispersal (Simon et al. 2002; Ebert 2005; Schröder 2005), such that cost-benefit calculations of sex are overwhelmingly driven by the demographic effects that arise from the different fates of dormant and directly developing offspring (Stelzer & Lehtonen 2016 demonstrate this for rotifers). A parent has the choice of asexual reproduction, which contributes to immediate local population growth, or of adding to the pool of sexually produced dormant eggs that may also disperse. Trade-offs between mean (arithmetic) fitness and its variance are particularly clear when the environment varies so much that some periods can only be survived in the dormant stage, and the timing of harsh conditions is difficult to predict. Asexual reproduction offers the potential for fast growth and competitiveness within a growing season, but sexual reproduction, which is the only means to produce dormant eggs, is ultimately necessary or the entire lineage fails to survive to the next season, should dormancy be needed in between. Sex is thus crucial for long-term survival of a lineage, but when should it be employed?

When sexes are separate, an added complication is that three types of offspring may be produced. In *Daphnia magna*, which we base our study on, dormant eggs can only be produced sexually. A mother can produce either daughters or sons asexually, or mate with males and produce dormant eggs provided with a protective shell, called an ephippium. Environmental

cues can induce both male production and sexual reproduction and thus the formation of dormant ephippia. Day length variation is an obvious cue of seasonal changes (Alekseev and Lampert 2001, Gilbert and Schröder 2004); for example, in northern Europe, shortening days that herald the approach of winter induce male production and sex (Roulin et al. 2013). However, hot spells in northern summers may also dry out populations in shallow water bodies. Additionally, if a population grows dense (via past asexuality), this can induce male production and sexual reproduction, e.g. due to increased competition or a reduced demographic cost of sex if high densities limit asexual growth (Gerber et al. *submitted*). An unanswered question is whether such responses are shaped by bet-hedging: does a winning strategy achieve its success by modulating its variance in fitness in a beneficial direction, even if mean (arithmetic) fitness declines (Starrfelt & Kokko 2012); and does this depend on whether cues of the timing of the season end are imprecise, indirect, or both? Our aim is thus to link sex allocation theory to the theory of bet-hedging in a potentially complicated setting, where equal investment into male and female offspring is not the *a priori* expectation (because of generational overlap with seasonal variation, Kahn et al. 2015, combining with female reproduction not being constrained by mate availability when asexuality is an option).

Consider a strategy that starts reproducing sexually early in the season, largely foregoing the chance to fill the local environment with asexually produced females. Within-season fitness is reduced and at any point there are only a few mothers available to produce ephippia (note that asexual *Daphnia* generations overlap *within* a season). However, early sex also has demographic benefits as it reduces the variance in fitness: whether or not the season ends early, some ephippia have been produced.

Conversely, a strategy that begins sexual reproduction only late in the season may yield some fitness benefits: a long period of asexuality means that various demographic costs of sex (Lehtonen et al. 2012) are avoided for several generations. Once sex begins, the lineage comprises many mothers producing ephippia. As a consequence, a late-sex lineage is very productive if the season lasts sufficiently long. However, if the season ends sooner than sex was 'scheduled' to begin, the entire lineage may still be asexual, with no ephippia produced to found the next generation. A risk-spreading strategy would then have been superior, producing at least some offspring equipped to survive the unfavourable season (Halkett et al. 2004, Tarazona et al. 2017).

While the above captures the essence of the problem, added complexity comes from the fact that sex requires males. In cyclical parthenogens, sex allocation decisions — what sex of offspring to produce — are not separate from which reproductive mode to invest in (sex or asex); production of males is pointless if no females are sexual in the near future. Currently, the only theoretical results for cyclical parthenogen sex allocation consider haplodiploid rotifers (Aparici et al. 1998, Serra et al. 2008). Their life-cycles differ from our case as male abundance in rotifers directly affects whether mictic (sexual) females produce sons or daughters (fertilized eggs develop into females). Also, these studies have not considered stochastic variation in season length, so do not comment on the relevance of bet-hedging. Our model considers male production as part of the bet-hedging framework needed to understand the timing of sex in cyclical parthenogens.

Methods

The *Daphnia* system

In most *Daphnia* species, sexual reproduction is coupled with the formation of dormant eggs (see Hebert 1981 for an exception). Female *Daphnia* are iteroparous with overlapping generations within a season, producing a clutch after every molt until death. Females can alternate between asexual and sexual reproduction throughout the season (Figure 1), producing three kinds of diploid offspring: asexually produced males and females, and sexually produced dormant eggs that develop after dormancy into females. Note that in *Daphnia* sex determination is environmental, not genetic, thus parthenogenetically produced offspring are clones of their mother irrespective of their sex (Ebert 2005). Asexual clutch sex ratios are strongly biased towards one sex (Booksmythe et al. *submitted*). Therefore, we assume that a female produces either a clutch of females or a clutch of males in each asexual cycle.

The cost of sex is complicated to compute for cyclical parthenogens (Stelzer and Lehtonen 2016) but is clearly substantial within a season, as sex leads to no directly developing offspring. Producing males likewise trades off directly with production of daughters who could increase the local density of females (each capable of eventual ephippia production). In addition, sexual reproduction in *Daphnia* entails a starkly reduced clutch size: females reproducing sexually produce only two eggs, while an asexual clutch can contain up to around 100 offspring (Ebert 2005, Gerber et al. *submitted*). Finally, sexually produced eggs need to be fertilized by a male before being enclosed in the ephippium, which is released into the environment upon the female's next molt; a female committed to producing an ephippium might fail to get these eggs fertilized, if no males were present

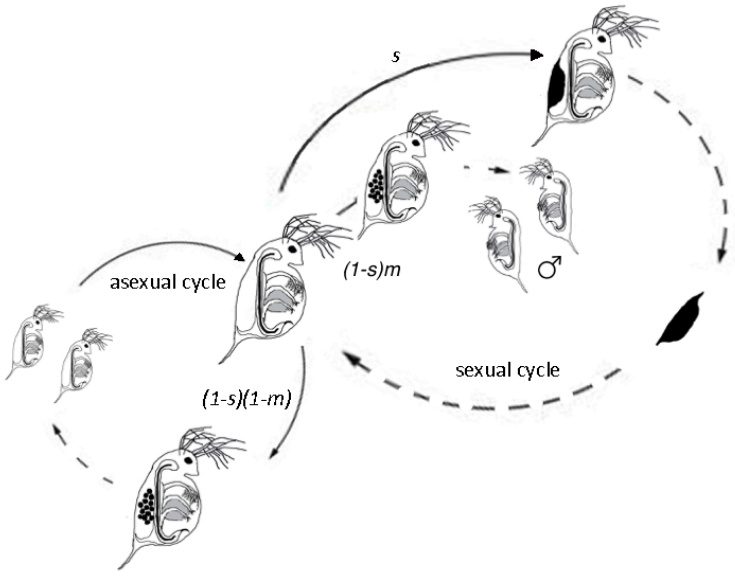

Figure 1 Daphnia life-cycle. Female Daphnia have three reproductive options in every breeding cycle. A female can produce asexual daughters with probability (1–s)(1–m), produce asexual sons with probability (1–s)m, or reproduce sexually and build an ephippium with probability s. The eggs that will be dormant inside an ephippium have to be fertilized by a male.

The model

The model is based on examining whether a mutant that uses a different schedule of male production and/or sexual reproduction from a resident strategy can spread in a variable environment. We first describe the fitness computations for each strategy. Thereafter, we describe how a genetic algorithm uses the relative fitness values of the strategies to update the best solution found so far, and creates a new set of potential competitor values to be tested. This section will also justify the use of a genetic algorithm. One 'round' of the genetic algorithm consists of evaluating the performance of several possible mutants, each of them evaluated with respect to all possible season lengths, against the currently best possible resident.

The fitness computations themselves represent a two-stage process, consisting of between- and within-season dynamics. First, we consider the *between-season dynamics*: what is the long-term fitness of a strategy with a known yearly sequence (distribution) of total ephippia produced? We use the approximation of geometric mean fitness (Starrfelt and Kokko 2012), justified when non-overlapping ephippial generations follow each other in time. Note that this implicitly assumes that all ephippia begin to contribute to populations in the immediately following season; this is realistic for *Daphnia* in shallow rock pools (Ebert 2005), where long-term deposits cannot accumulate. It is also a good approximation for other systems as long as contributions from older sediments remain negligible. We next compute the *within-season dynamics* of accumulated ephippia production for each strategy: what is the probability of a given strategy having produced 0, 1, …, ephippia by the time the season ends? Our modelling tracks the accumulating ephippia production within a season for any given strategy. The realized accumulated number depends not only on the strategy but also on season length, modelled as a random variable.

Daphnia locally adapt to day length (Roulin et al. 2013) but reproductive decision-making can also be density-dependent (Kleiven et al. 1992, Berg et al. 2001, Gerber et al. *submitted*). We therefore make two alternative assumptions about how *Daphnia* infer time in the season: first, that they have perfect information about the time already elapsed (direct cue); second, that this information is imperfect and the only cue available is current population density (indirect cue). Additionally, we consider two scenarios where population growth does, or does not, experience density-dependence. The density-independent scenario is simpler (and might be accurate for a population that cannot saturate in a large water body), while the inclusion of density-dependence is typically more realistic. We model the former (density-independent growth) by assuming each asexual clutch leads to c mature individuals, while in the latter case the number of maturing individuals declines with population density. This yields a 2 × 2 table of assumptions such that density is allowed to impact the population as a cue only, as a factor influencing population growth only, both, or neither (Table 1).

The definition of bet-hedging is satisfied when a strategy reduces mean (arithmetic) fitness as well as the variance in fitness. As reductions cannot be evaluated unless there is a baseline, we require a hypothetical non-bet-hedger for comparison (Li et al. in press). This hypothetical non-bet-hedger obeys the same rules as above, but maximizes arithmetic rather than geometric mean fitness. We present calculations based on this hypothetical organism to evaluate whether the timing of sex can be considered a bet-hedging trait.

The calculations below are, wherever not specifically indicated to differ, identical for each version of the model.

Table 1 The 2 x 2 assumption sets.

Population growth	Cue: Direct	Cue: Indirect
Density-dependent	**Direct cue, constant *c*** $s_t = s_t$; $m_t = m_t$ $c_t = c$	**Indirect cue, constant *c*** $s_t = s(N_t)$; $m_t = m(N_t)$ $c_t = c$
Density-independent	**Direct cue, density dependent *c*** $s_t = s_t$; $m_t = m_t$ $c_t = c(N_t)$	**Indirect cue, density dependent *c*** $s_t = s(N_t)$; $m_t = m(N_t)$ $c_t = c(N_t)$

1. Between-season dynamics

We base our model on the life history of *Daphnia*, confining our view of the benefits of sex to its strong demographic effect in this system: sex is required to survive harsh conditions, because it is the only means to produce dormant eggs.

The probability that the season ends after T breeding cycles is denoted p_T, and all active individuals die at this point while ephippia produced during the season carry on their dormancy. The fitness of a clone is defined by the geometric mean of ephippium production over all possible season lengths. We assume all ephippia hatch in the season following their production, irrespective of the precise timing of their production. A clone's fitness W is the geometric mean of the total number of gene copies, G_T, in dormant ephippia at the end of a season, computed for each possible season length t and weighted by the probability that each season length occurs, p_T (we assume that the maximum season length is T_{max}). Computationally it is easier to work with the logarithm of fitness

$$ln\, W = \sum_{T=T_{min}}^{T_{max}} p_T ln\, G_T \qquad (1)$$

Theoretically, the probability distribution p_T can take any shape. We explore the effect of uniformly distributed probabilities where p_T takes the same value for all integers between $T_{\min}$ and $T_{\max}$ (we avoid setting $T_{\min} = 1$ as seasons this short preclude the maturation of asexually produced young). Varying the difference between $T_{\min}$ and $T_{\max}$ allows us to explore different season predictabilities, from a highly predictable scenario where the season can only end at $T_{\max}$ to highly unpredictable scenarios where the shortest seasons, of length $T_{\min}$, are far shorter than the longest seasons that last $T_{\max}$ breeding cycles. We have chosen a uniform distribution to achieve a range of scenarios from no variance in season length to very high

variance. Note that the method of analysis could be applied to any desired shape (e.g. bimodal) of season length distributions; it would simply involve entering a desired set of p_T values in eqn. (1).

2. Within-season dynamics

The within-season dynamics specify what females do (using the options described in Figure 1) at each of the possible breeding cycles from $t = 1$ to $t = T$ (the end of the season, which can go up to T_{max} or end sooner), and quantifies the number of ephippia that accumulate up to the time point when the season ends. Asexual reproduction results in lineages sharing the same genotype, which defines the reproductive strategy. Genotypic similarity does not affect the strength of competition between clones. Different clones use strategies that differ in the probability of reproducing sexually at time t (denoted s_t), and in the conditional probability of producing males if reproduction was not sexual (denoted m_t). Thus, at each time point a female's three options are an all-female clutch (probability $(1–s_t)(1–m_t)$), an all-male clutch (probability $(1–s_t)m_t$), or an ephippium (probability s_t). The difference between the direct cue strategy set and the indirect cue strategy set is that s_t and m_t are determined by time t in the former, but are a function of population density at time t in the latter: $s_t = s(N_t)$ and $mt = m(N_t)$. Individuals within a clone apply these probabilities independently, thus one female may use asexual reproduction while another reproduces sexually.

Sexual clutch size is fixed to 2 eggs. We also need to account for the possibility that the number of gene copies present in these eggs, produced by a certain timing strategy, differs: if the male as well as the female are from the same clone, the genetic representation is doubled compared to outbreeding. Here we deviate from 'pure' invasion analysis of a rare mutant, where mutants are assumed so rare that they do not encounter each other. Our rationale is that should the resident population produce no males, the ability of a mutant clone to reproduce sexually depends on its own male production, and we want to include this effect. Asexual clutch size varies and is typically much larger than 2. We denote the number of individuals that mature from one clutch as c (c captures the effect of clutch size as well as juvenile survival). Where population growth is density-dependent, we assume a sigmoid relationship between c and population density. This relationship is approximated from field data (Gerber et al. *submitted*) with c ranging from the mean asexual clutch size of the largest 10% of measured clutches at low densities to ~ 0 at very high densities (Figure S1). Where population growth is independent, we keep c constant. When carrying capacity is reached, individuals can still reproduce sexually, as sexually produced eggs do not contribute to current population density. We assume equal survival for male and female clutches.

We follow the dynamics of a rare mutant clone in a population otherwise consisting of a resident strategy. Mutants only differ from residents with respect to timing, thus our model operates in the absence of any other differences in competitive ability. Below, we use s_t and m_t to refer to the strategy set regardless of whether cues are direct (time) or indirect (density); the difference in interpretation is that if population densities are the same at two time points, the corresponding s_t and m_t values may differ in the direct cue setting but must be the same if cues are indirect. We use the superscript ^{r} when referring to the resident strategy, contrasting with the mutant strategy (denoted by ') whose fitness we evaluate.

We first calculate the dynamics of the resident population strategy, which determines the environment in which a mutant strategy's fitness is evaluated. Each female following the res-

ident strategy reproduces sexually with probability s_t^r at time t, and produces males with probability m_t^r if reproducing asexually. To compute the density of mature individuals in the resident population at time t, we consider that asexually produced males and females take two breeding cycles to mature. The mortality of mature individuals is assumed to equal d_f (females) or d_m (males), when measured over one breeding cycle.

The number of mature females at time t (F_t^r) equals the newly matured females that were produced at time t–2, plus the number of surviving previously mature females (F_{t-1}^r):

$$F_t^r = F_{t-2}^r c(1 - s_{t-2}^r)(1 - m_{t-2}^r) + F_{t-1}^r(1 - d_f) \quad (2a)$$

The equivalent male equation reads:

$$M_t^r = F_{t-2}^r c(1 - s_{t-2}^r)m_{t-2}^r + M_{t-1}^r(1 - d_m) \quad (2b)$$

The sequence of F_t^r, M_t^r values forms the environment in which a mutant's fitness is evaluated. Mutant dynamics (F_t' and M_t') are computed analogously to eqns. (2a-b),

$$F_t' = F_{t-2}' c(1 - s_{t-2}')(1 - m_{t-2}') + F_{t-1}'(1 - d_f) \quad (3a)$$

$$M_t' = F_{t-2}' c(1 - s_{t-2}')m_{t-2}' + M_{t-1}'(1 - d_m) \quad (3b)$$

To compute mutant fitness, we assume the population starts with one mutant among a much larger number of resident females (999 in our examples, bringing the total population to 1000; note that in *Daphnia*, all individuals hatching from ephippia are female). In practice, the ratio of mutants to residents matters little for the outcome.

Sexual reproduction requires that males are present in the population. Whenever $M_t^r + M_t' > 0$, the total number of ephippia produced at time t by the mutant clone equals $F_t' s_t'$. Each ephippium contributes, on average, fitness $1+M_t'/M_t^r$ to the clone that produced it (M_t'/M_t^r being the probability that the father belongs to the same clone); simultaneously, the resident population produces $F_t^r s_t^r$ ephippia, and each of these contributes, on average, M_t'/M_t^r units of fitness to the mutant clone, via paternity. Thus the fitness accrued by the mutant strategy at time t is

$$g_t' = F_t' s_t'(1 + M_t'/M_t^r) + F_t^r s_t^r M_t'/M_t^r \; if \; M_t^r + M_t' > 0 \; 0 \; otherwise \quad (4)$$

If the season lasts until $t = T$, total fitness accumulation equals

$$G_T = \sum_{t=1}^{T} g_t \quad (5)$$

G_T is inserted into the between-season dynamics equation (eqn. 1). A full evaluation of the value of eqn. (1) requires as many calculations of eqn (5) as there are possible season lengths, T. The resulting G_T for each possible T is then weighted with the probability p_T that this length occurs in nature (eqn 1). Note that this approach does not involve replacing the resident with a mutant as soon as the latter has a superior G_T value in any specific season, which could erroneously lead to assigning high success to a strategy with no long-term prospects. Instead, the winner (based on eqn. 1) is only determined once success has been computed over the entire distribution of season lengths that can occur in nature.

3. Genetic algorithm

In principle, the above steps are sufficient to find an overall winner. Practically, however, finding the fitness-maximizing values for s and m for every time point t is challenging for

several reasons. First, while *s* and *m* can have independently evolving values at every breeding cycle or population density (depending on model version), this may conflict with the general notion that we might not expect perfectly fine-tuned evolutionary responses to minor variations in the environment (McNamara et al. 1997, McNamara and Houston 2009). Second, the chosen strategy at time t can influence future time points (e.g. a diminishing proportion of males produced at time t are still available in future breeding cycles). Third, the optimal strategy depends not only on the mutant clone's behavior, but also on the behavior of the resident population: e.g., male production pays off less well if there are no outbreeding opportunities because the resident population produces no ephippia, and sex is possible without producing males if others produce them, but requires own male production if not. Finally, we must calculate the geometric mean as a fitness measure for every possible season end. Based on the complexity of the situation, we do not seek analytical solutions but use a genetic algorithm that follows haploid sexual reproduction. Note that we choose a genetic algorithm not to simulate chromosomes of *Daphnia* accurately, but as a tool to find solutions to a complex life-history problem.

The algorithm

First, we create a resident strategy s_t^r and m_t^r for every breeding cycle by drawing uniformly distributed random numbers between 0 and 1 for every t. This is simple for the direct cue scenario, but requires that s_t^r obeys a functional form $s^r(N_t)$ in the indirect cue scenario. For the latter we group the logarithmic population density into 100 categorical bins with logarithmically spaced borders, such that the first bin contains all log densities between 0 and 1, the second bin between 1 and 2, …, until 100 (the largest population density our model can handle is e^{100}, sufficiently large to be never reached). $s(N)$ is assumed equal for the range of population densities in one bin. In the next step, nine mutant strategies s_t' *and* m_t' (or $s'(N_t)$ and $m'(N_t)$) are created by adding normally distributed random numbers to the resident strategy. Values below zero are set to zero and values larger than one are set to one, so that $1 > s_{bin}', m_{bin}', s_t',\ m_t' > 0$.

Using eqns. (2-3) we can calculate the dynamics of the resident and mutant strategies and the fitness of each strategy; this is given by eqn (1) except for the case of a hypothetical non-bet-hedger, for which (1) is replaced by

$$W = \frac{1}{T_{max}} \sum_{T=1}^{T_{max}} p_T G_T \qquad (6)$$

We thereafter keep the two strategies that yield the highest fitness, and the resident strategy. These three strategies offer three options for *s* and *m* for each breeding cycle (or density bin). The algorithm creates nine recombinant strategies by randomly choosing, for each breeding cycle (or density bin), a value with a probability 1/3 from any of the three options available. Additionally, with probability p (independently applied for each cycle or bin of each recombinant strategy), the recombinant may take its value from a temporally adjacent breeding cycle (or density bin). This speeds up the optimization process because well-performing actions at time T can spread to temporally neighbouring points. Strategies at the first and last breeding cycle (or density bin) only have one adjacent strategy to copy from.

Finally, the fitness of the original resident and the recombinant strategies are calculated as described for the mutant strategies. The strategy with the highest fitness becomes the new resident strategy from which mutants are created in the next round of the algorithm.

This algorithm optimizes the values for s_t and m_t at every breeding cycle within a season. Assuming convergence, this yields the approximately optimal strategy of the timing of sexual reproduction and male production. For each scenario we ran the algorithm until the optimization criterion (eq. 6) remained unchanged for 100 iterations. This criterion was reached after a mean of 6238.8 to a mean of 10358 rounds, depending on scenario. Instead of averaging independent runs, we present the results of the best-performing strategy of 10 independent runs, as we are able to compare in absolute terms the performance of strategies produced by a genetic algorithm, and finding a better one makes a previously found, less well-performing strategy obsolete.

Results

Direct cue of time, density-independent population growth

Where females have information on how far the season has progressed, and population growth is density-independent, presents the simplest case of all our model versions. If we additionally assume that the season end is perfectly predictable (Figure 2a), females only invest in sexual reproduction very late: it is more efficient to reproduce asexually unless the season end is imminent. To be precise, females switch to sexual reproduction two breeding cycles before the season ends (Figure 2). As we assume that it takes two breeding cycles for directly developing offspring to mature, it is optimal to switch to sex when asexually produced daughters no longer have time to mature and contribute to reproduction. Similarly, because males also need two breeding cycles to mature, and successful sex requires males, they are produced two breeding cycles before sex is induced (Figure 2a). Predictable conditions therefore lead to late sex, and the situation additionally recovers, to an approximate degree at least, the familiar principle of equal investment in sex allocation (West 2009): during male production (time steps 27 and 28 in Figure 2a), the sex ratio of newly produced offspring deviates only slightly from 50%. The deviation is in the direction of more male production (>50%), and it is easy to understand: any male produced arises during these time steps, while females may have been produced earlier, and some of these females may still be alive when sexual reproduction commences.

Increasing uncertainty leads to a far wider spread of sex and male production (Figure 2b-c), and to some inaccuracy of the 'best' algorithm as a small fraction of males are produced later than they can possibly mature (Figure 2b,c at times 29 and 30; note that very few generations actually experience seasons this long, which helps to explain the approximate performance of the algorithm at this end). Any straightforward expectation of equal investment becomes unjustified, a result known from sex allocation theory when scenarios involve overlapping generations and sequential decisions with mortality occurring in between (West 2009, Kahn et al. 2015). As an additional complication, the facultative nature of sex means that males can only hope to fertilize eggs when females commit to sexual egg production, thus we expect deviations from 1:1 sex ratios mostly in the downwards (fewer males) direction.

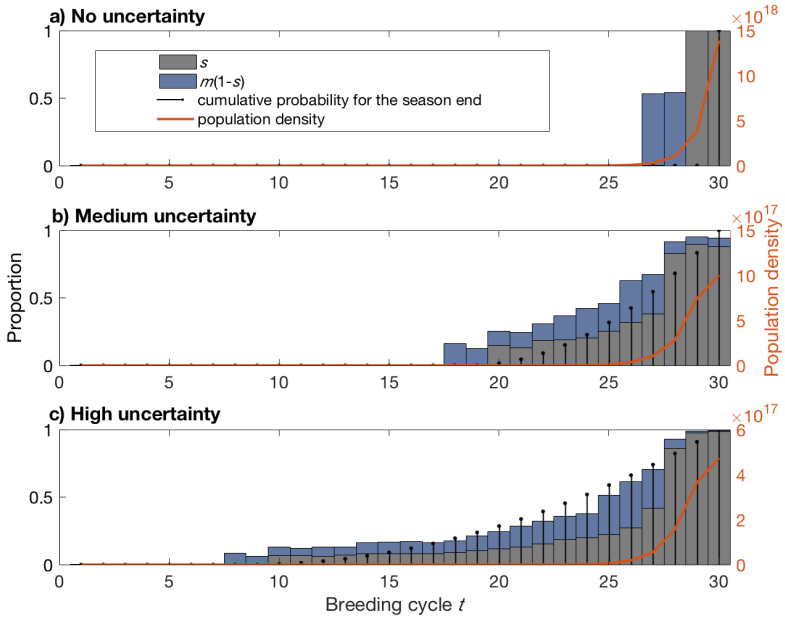

Figure 2 Direct cue, density-independent population growth. The optimal proportions of sexual reproduction, s, and male production $(1–s)m$, at every breeding cycle in a season following the assumptions in the lower left corner of Table 1;. The remaining white area therefore indicates female production, $(1–s)(1–m)$. Panels illustrate three different predictabilities of the season end (the cumulative probability of which is indicated with vertical lines); we show the best strategy of 10 independent runs of the genetic algorithm. Panels show: **a)** the season will predictably end at $T_{min} = T_{max} = 30$; **b)** there are 11 possible end times between $T_{min} = 20$ and $T_{max} = 30$; **c)** there are 21 possible end times between $T_{min} = 10$ and $T_{max} = 30$. All panels use $d_f = d_m = 0.05$, $c = 10$.

The model confirms this expectation, and predicts that uncertain season lengths make the production of males and sexual eggs overlap, with an increase that is shallower than the cumulative probability that the season has ended by time t. The common feature between all cases is that male production commences precisely two breeding cycles before the production of sexual eggs, reflecting our assumption of a fixed time to maturity. While conceptually clear, this density-independent model scenario is unrealistic for long seasons, as populations reach unrealistically high densities (10^{17} – 10^{19} individuals).

Direct cue of time, density-dependent population growth

Introducing density-dependence retains many of the patterns of the density-independent model while keeping population densities within a realistic range. Male production precedes sexual reproduction, and increased unpredictability of the season length broadens the time over which sex occurs. The main difference from the density-independent model is that asexual female production ceases as soon as carrying capacity is reached (Figure 3). If adult mortality is low, this cessation may be permanent, and adult population size gradually declines over the remainder of the season (Figure 3b-c) or, in the predictable season end case, even earlier (Figure 3a). During this time, existing females simply release ephippia – as males die at the same rate as females they are mostly still available to fertilize the ephippial eggs.

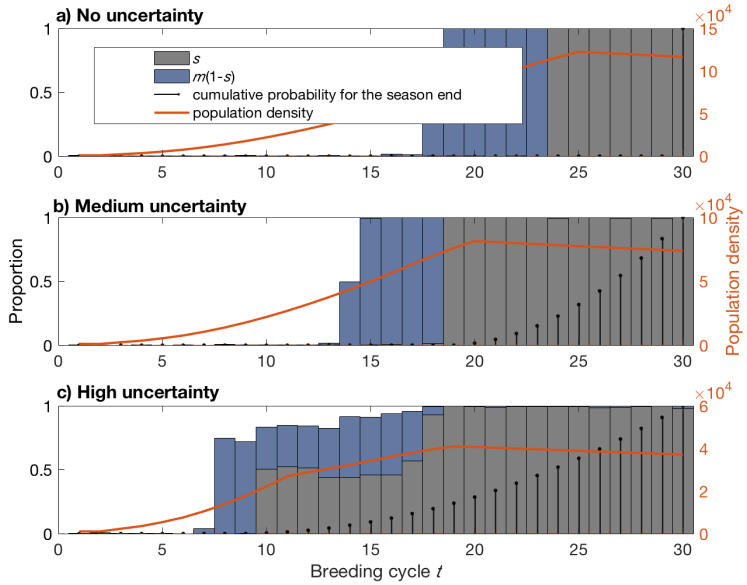

Figure 3 Direct cue, density-dependent population growth. The same notation and parameter values as in Figure 2, with assumptions following the top left corner in Table 1, where recruitment c obeys the function exp(4 + (–5 – 4)/(1 + exp((–0.3)(ln(density) – 8)))) (see Figure S1).

The above assumes low mortality, such that the adult population size decays slowly in the absence of new, directly developing offspring. If adult mortality increases and if the season length is very unpredictable (Figure S2), the solution changes to an early peak of sexual reproduction (preceded by male production) during which female production does not completely cease, followed by a switch back to the predominant production of directly developing females, which in turn precedes the final return to sexual reproduction (again with male production first). In this setting, some ephippia will have been produced by the time that the season end is possible but unlikely, while most sex happens later when the cumulative probability of the season having ended becomes appreciable. The population density shows, correspondingly, two peaks (Figure S2c).

Indirect cue (density is a cue for time), density-independent growth

There is relatively little difference between populations with access to direct cues (Figure 2) and those that rely on the indirect cue of population density (Figure 4). However, the latter case shows mild signs of the algorithm having produced only an approximate solution: in Figure 4a, male production begins three breeding cycles before eggs requiring fertilization appear. This may reflect computational inaccuracies as we were forced to assume identical responses to all within-bin population densities that occurred in a population.

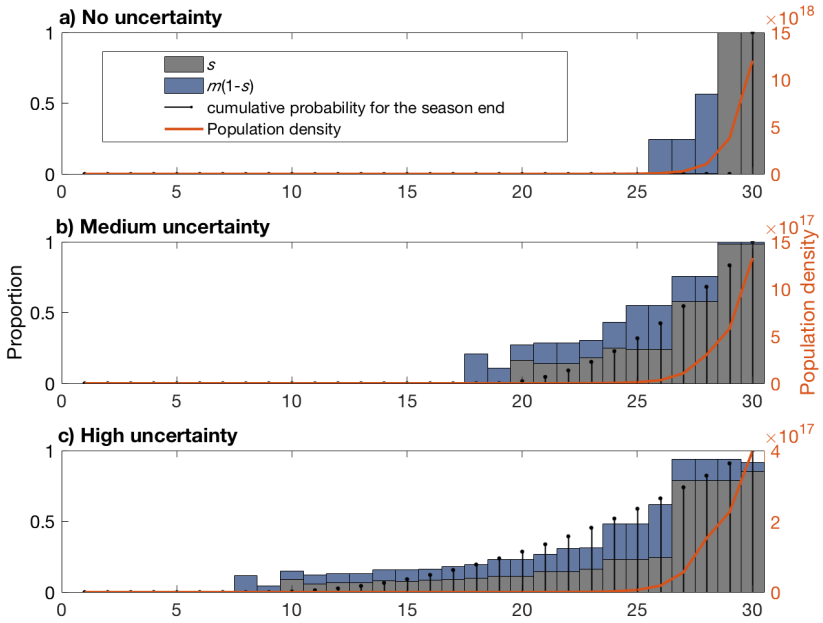

Figure 4 Indirect cue, density-independent population growth. The same notation and parameter values in Figure 2, but with assumptions following the top right corner of Table 1.

Indirect cue (density is a cue for time), density-dependent population growth

When population growth is density-dependent and density itself acts as a cue to switch between reproductive modes, we find qualitatively the same pattern as before: density-dependent population growth greatly broadens the time over which sex occurs (Figure 5) compared with the density-independent scenario (Figure 4). If adult mortality is low, the asexual production of directly developing females is low over much of the season (Figure 5). Higher adult mortality creates a stronger need to 'replenish' the population of adult females should the season continue (Figure S3), and the details of season length then determine if population density shows cyclic behaviour, or a slow increase or decline (Figure S3). At high and low season length predictabilities, male and sexual egg production overlap, and population density stays relatively constant (Figure 5a,c, Figure S3a,c). Interestingly, intermediate season length predictability can produce density cycles, where peaks correspond to the highest prevalence of sex (Figure 5b, Figure S3b). Frequent sex leads to a decline in population size (adult mortality combines with little asexual offspring production), which in turn induces asexual production of males and females. The sex allocation pattern during this phase shows fewer males if adult mortality is higher (contrast Figure S3b with Figure 5b).

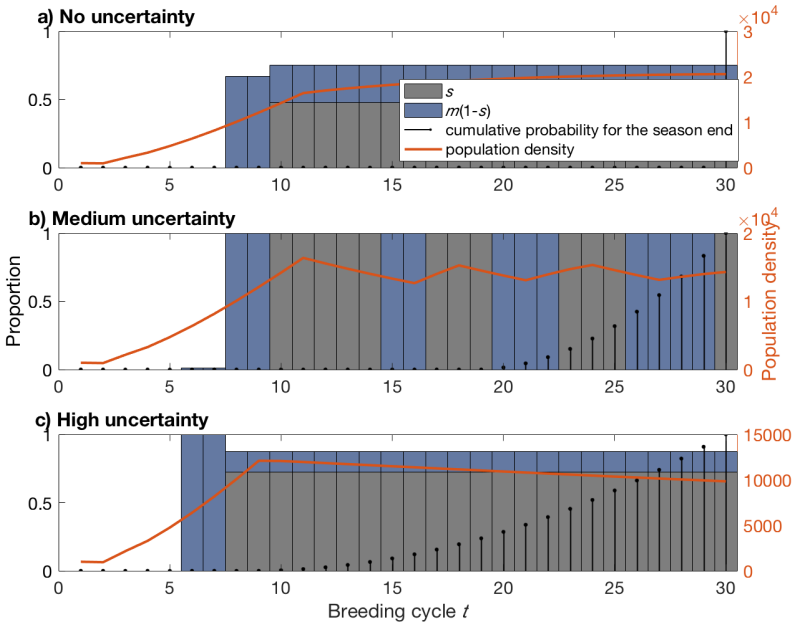

Figure 5 Indirect cue, density-dependent population growth**.** The same notation and parameter values as Figure 2, with assumptions following the lower right corner in Table 1, and thus assumptions regarding recruitment c follow those of Figure 3.

Evaluation of bet-hedging

To evaluate whether a strategy selected to optimize geometric mean fitness (measured as total ephippia production over multiple seasons) meets criteria for bet-hedging, we compared the arithmetic mean and variance in ephippia produced by this strategy with the mean and variance produced by a hypothetical non-bet-hedger, selected to optimize arithmetic mean fitness. Although this non-bet-hedger cannot persist in nature if it produces 0 offspring in any given season, it forms the necessary baseline allowing us to evaluate the fitness moments that helped the evolved strategy express its demographic superiority. Relative to the non-bet-hedger, we expect a decrease in both the arithmetic mean fitness and the variance in fitness of the strategy optimizing geometric mean fitness, if this strategy is a bet-hedger.

Absolute fitness means and variances differed greatly across model assumption sets (Figure 6), however bet-hedging criteria were satisfied in all environments (which generally spanned a larger range of potential season lengths than the examples shown in Figures 2-5). The difference in variance was not always of the same magnitude as the difference in mean (lines in Figure 6 are not parallel), and the absolute magnitude of the differences depended strongly on the unpredictability of the environment. As expected, a shorter period of uncertainty (a late T_{min}) led to a smaller difference in either fitness moment between the bet-hedger and the non-bet-hedger.

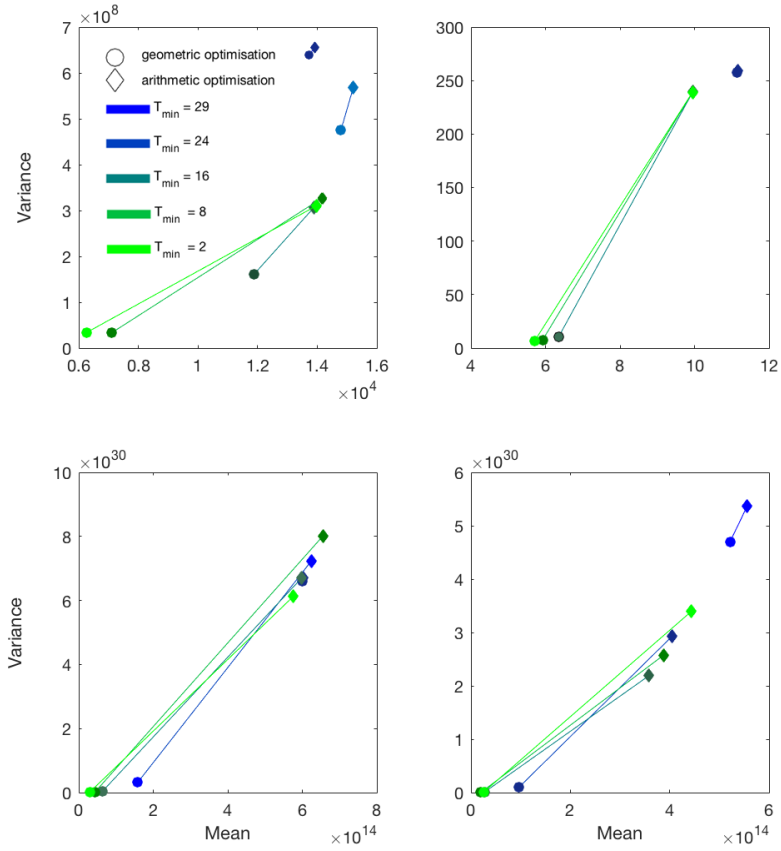

Figure 6 Bet-hedging. Comparison of the (arithmetic) mean and variance in ephippia production for strategies selected to optimize either the arithmetic mean (diamond) or the geometric mean (circle) ephippia production. The four tested scenarios correspond to those listed in Table 1. All panels use $d_f = d_m = 0.05$ and $T_{max} = 30$, with recruitment c being either fixed at 10 (top row) or density-dependent as in Figure S1 (bottom row).

Discussion

Strategies of sex allocation and reproductive mode in cyclical parthenogens are poorly understood, especially when environments are unpredictable. Our models show that for facultatively sexual organisms, spreading the use of sexual reproduction, and with this the production of dormant eggs, over several breeding cycles can be adaptive in unpredictable environments, and this strategy satisfies criteria for bet-hedging. When season length is unpredictable, the induction of sexual reproduction is driven by the temporal probability distribution of the length of the season, with important modifications that depend on the seasonal cues available and on whether recruitment is density-dependent.

When the end of the season is predictable, sexual production of dormant eggs only occurs shortly before the season actually ends. In all other environments, sexual production of dormant eggs is a risk-spreading strategy, with some ephippia produced as soon as there is some risk that the season might end, and male production beginning strategically as long beforehand as is needed for the first male generation to mature. Our result that sex is spread more widely when season length is more variable is consistent with models based on other cyclical parthenogen life histories, such as those of aphids, where optimal investment in sexual reproduction is proportional to the variance in the season end (Halkett et al. 2004). A

gradual and precocious induction of sex in uncertain environments was also described in a general model of cyclical parthenogens, where sexual reproduction is linked with dormancy (Spencer et al. 2001).

These earlier studies, however, did not link their findings to literature on sex allocation. In this context, cyclical parthenogens present another set of problems (Aparici et al. 1998, Serra et al. 2008). For example, is there a sense in which equal allocation to males and females is maintained? In some qualified sense the answer may be yes: in monogonont rotifers, sexual females are a specific subset of all females, and male production may evolve to match their number (Serra et al. 2008). However, when a specific female does not have to commit to sex (e.g. in *Daphnia*, females can switch back and forth between reproductive modes), it is less clear whether one can expect any general insight regarding total investment. This is due to the dynamic nature of sex allocation, where males produced in different time steps may co-exist and compete for females — only some of which offer fertilization opportunities. In alignment with other situations where a sequential nature of interactions (including mortality) can blur an expectation based on investments (Kahn et al. 2015), our results highlight that near-equal sex ratios are only produced in rather exceptional circumstances, where all females are about to turn sexual very soon.

A more general question, then, arises: should male production take place mainly before sexual reproduction begins, or co-occur with sex to enable replenishment of the male population? We find the former solution more often if individuals have direct cues of how late it is in the season, allowing for more precisely orchestrated timing of each category of individuals. The alternative, where some females produce male clutches while others produce females and yet others develop eggs requiring fertilization, tends to associate with indirect cues, although not categorically. Indirect cues can also lead to temporal separation of male production and sex, if at high density sex becomes so prevalent that the current population declines. Once it has decreased below a threshold, the best strategy switches to replenishing the adult population, and male production once again anticipates future mating opportunities (cf. Kahn et al. 2013).

How well do our predictions match what is found in nature? In predictable habitats we show that sexual formation of dormant eggs should be delayed, with a peak towards the predetermined season end or at high population densities. Empirical studies find that *Daphnia* populations in predictable habitats, such as permanent water bodies in seasonal, temperate environments, produce ephippia only at the end of the season (Galimov et al. 2011), whereas *Daphnia* from shallow ponds at northern latitudes, which freeze over winter and occasionally dry out in summer, produce ephippia throughout the season (Altermatt and Ebert 2008, Gerber et al. *submitted*, Roulin et al. 2013). While studies that consider the timing of male presence in a population tend to focus on the co-occurrence of males with sexually reproducing females, rather than the timing of male production *per se*, many of these detect males substantially earlier than the appearance of ephippial females (e.g. Galimov et al. 2011). Our own empirical results (Gerber et al. submitted), as well as a previous study (Innes 1997), track clutch sex ratios over time in natural *Daphnia* populations, additionally show that male production continues throughout the season, overlapping with ephippia production.

Likewise, our results on density appear well supported by data. When we included density-dependent recruitment in the model, sex was induced as soon as population density peaked, in some cases showing multiple peaks. One interpretation is that the direct demographic cost

of sex, relative to asexual reproduction, is reduced at high population densities because asexuality becomes inefficient. In several cyclical parthenogens, population density is an important predictor of the frequency of sex (*Daphnia*: Gerber et al. *submitted*, Carvalho and Hughes 1983, Kleiven et al. 1992, rotifers: Serra et al. 2008, Stelzer and Snell 2003). In our model, providing the algorithm with information on population density was sufficient to adapt the propensity for sex to the temporal pattern of season length, in the absence of direct information about the season's progression.

For density cues to work in a bet-hedging framework requires a correlation between time (since the beginning of the season) and population density. In seasonal populations with few founders at the beginning of each season and a large population later on, such a correlation arises fairly automatically. Interestingly, with sufficient adult mortality, our model showed the possibility of endogenous population cycles: mortality exceeded recruitment during high density (and consequent high sex) periods, which were followed by periods of lower density which, in turn, led back to asexuality and an increase in short-term population growth. Determining whether observed cycles in nature result from this process requires more fine-scaled analysis than the mere observation that sex peaks at high density, although this observation is consistent with the above reasoning. High rates of sex necessarily lead to lower population growth when sexual offspring cannot recruit and mature in the near future (Stelzer 2012), so the demographic features of our model should apply to many systems. More details would be welcome from natural systems; for instance, we did not allow individuals using indirect cues to perceive the density of males and females separately, even though this may be possible for *Daphnia* (Booksmythe et al. *submitted*).

Regardless of the details of our model variants, we found the timing of sex in cyclical parthenogens to represent a bet-hedging strategy, where mean fitness is traded off against a reduction in fitness variance. Empirical studies have also shown that the formation of dormant stages can represent bet-hedging when the season is unpredictable or cues are uncertain (Bradford and Roff 1993, Graham et al. 2014, Furness et al. 2015). It is interesting to reflect on the classic examples of bet-hedging that consider the optimal duration of dormancy in plants (Cohen 1966) or animals (Hanski 1988). Incorporating the possibility of additional bet-hedging in ephippial dormancy duration might influence the optimal strategy for sex induction in our model, because failing to produce ephippia in one season does not guarantee lineage extinction when ephippia from past seasons can hatch in the future. However, our model should provide a good approximation for systems where long-term accumulation of dormant stages is impossible or when reaching hatching conditions again becomes highly unlikely, due to e.g. habitat structure or high predation risk. Earlier theoretical work investigating the interaction of the timing of sex with the hatching fraction of eggs (Spencer et al. 2001) shows low hatching (interpretable as high bet-hedging) covaries with situations that lead to high variability in within-season reproductive output. Thus, the need to bet-hedge within a season might not readily disappear even if organisms invest in another bet-hedging strategy (delayed hatching).

When sexual reproduction is linked to an ecological function (such as dormancy), which is crucial for survival through some range of the temporal variation in an environment, cyclical parthenogenesis is always superior to a pure asexual strategy. This upholds the 'best of both worlds' view of the benefits of facultative sex: periods of fast demographic growth (avoiding costs of sex) alternate with periods of investment in sex (as a long-term survival strategy). Often, facultative sex is said to offer the 'best of both worlds' in a different setting: reaping

the genetic benefits of sex while (in most generations) avoiding its costs (D'Souza and Michiels 2010, Burke and Bonduriansky 2017). Our model focuses on the marked differences in the demographic consequences of sexual and asexual reproduction, which exist whenever sex and dormancy are linked, and does not consider potential genetic benefits of sex. Although genetic consequences of sex are known to scale non-linearly with the frequency of sex (Green and Noakes 1995), accounting for this would probably not change the timing of sex much in our model, because all founding individuals at the season's beginning are sexually produced, and offspring produced thereafter are their clones (leaving little scope for direct competition between sexual and asexual genotypes which could be impacted by processes considered by Green and Noakes 1995).

Our model is based on the premise that sex and dormancy are tightly coupled, an assumption that we base on findings that this is indeed widespread in organisms as diverse as Cladocerans (Tessier and Caceres 2004, Wittmann et al. 2011), aphids (Simon et al. 2002), rotifers (Carmona et al. 2009), and oligochaete worms (Schierwater and Hauenschild 1990), as well as many plants, where resistant seeds are produced sexually. While our model (and that of Spencer et al. 2001) is silent on *why* this link exists, an obvious next question is whether there is an adaptive reason to expect dormant forms to be sexually produced, while directly developing offspring result from parthenogenesis — especially because some exceptions exist (for an obligately asexual *Daphnia* lineage that can produce dormant stages asexually see Innes et al. 2000; bdelloid rotifers behave similarly, Caprioli & Ricci 2001). We will address this question in our future work, by relaxing the assumption of a pre-existing constraint and by considering a wider variety of potential benefits of sex (Gerber and Kokko in prep.).

In sum, we show that spreading sexual reproduction over several breeding cycles within a season is expected to evolve in unpredictable environments such that mean arithmetic fitness is traded off against variance in offspring survival (via dormancy) across seasons. This confirms that bet-hedging between dormant vs. directly developing offspring represents an important mechanism shaping the timing of sex. Additionally, population densities can influence the evolution of the induction of sex and male production in two ways. First, density can act as a cue for time in the season when no direct cues are available. Second, in populations with density-dependent growth, the demographic cost of sex (foregoing direct reproduction) is reduced when recruitment of directly developing asexual offspring is hampered by high population density. In some cases, the best responses to uncertain cues lead to endogenously produced population cycles.

Acknowledgements

We are grateful to Dieter Ebert for stimulating discussions and to two anonymous reviewers as well as Russell Bonduriansky for helpful comments. This project was funded by the Academy of Finland (Finnish Centre of Excellence in Biological Interactions Research) project number SA-252411 (to HK).

Data accessibility

The code for this project is available on GitHub: https://github.com/nigerb/DSRCode

Authors' contributions

NG, IB and HK conceived the study; NG and HK constructed the model and analysed the data NG, IB and HK wrote and edited the manuscript; all authors revised the manuscript and gave final approval for publication.

Appendix Chapter II

Figure S1 Density-dependence of c In the density-dependent scenarios the recruitment *c* obeys the function exp(4 + (–5 – 4)/(1 + exp((–0.3)(ln(density) – 8)))).

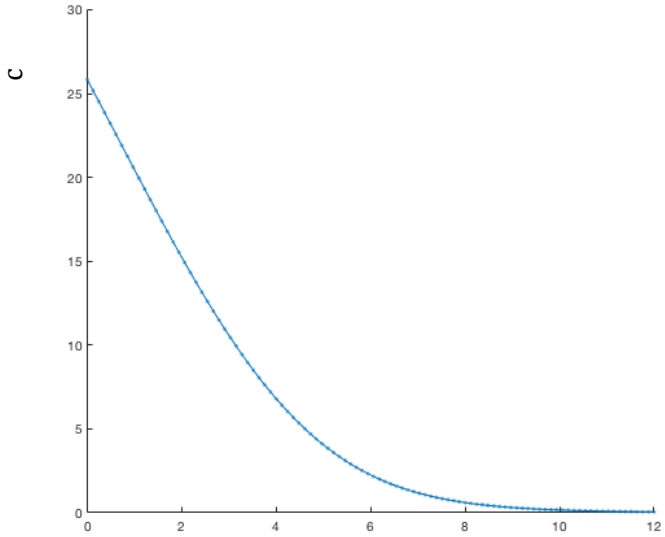

Figure S2 Direct cue, density-dependent population growth and high mortality. The figure is as Figure 3, but with $d_f = d_m = 0.1$.

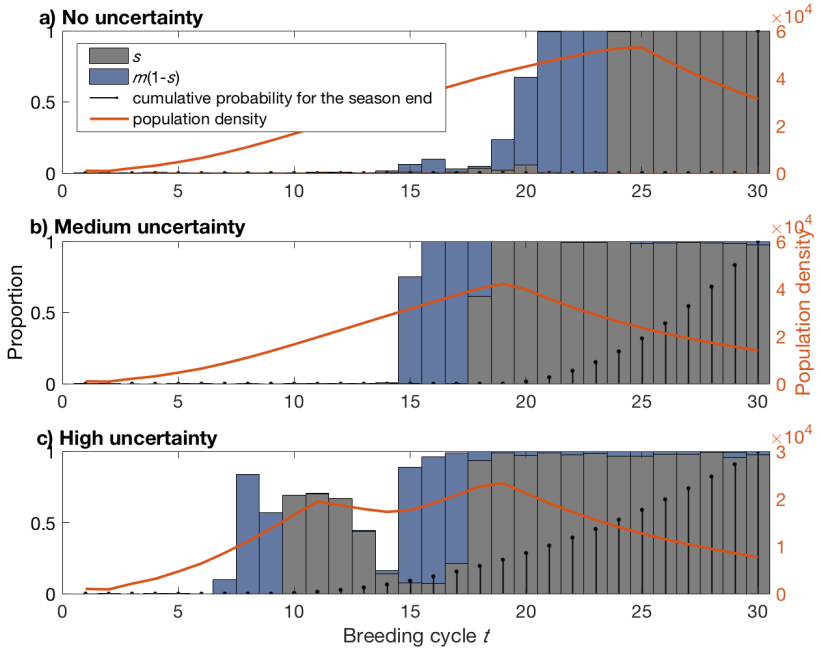

Figure S3 Indirect cue, density-dependent population growth and high mortality. The figure is as Figure 5, but with $d_f = d_m = 0.1$.

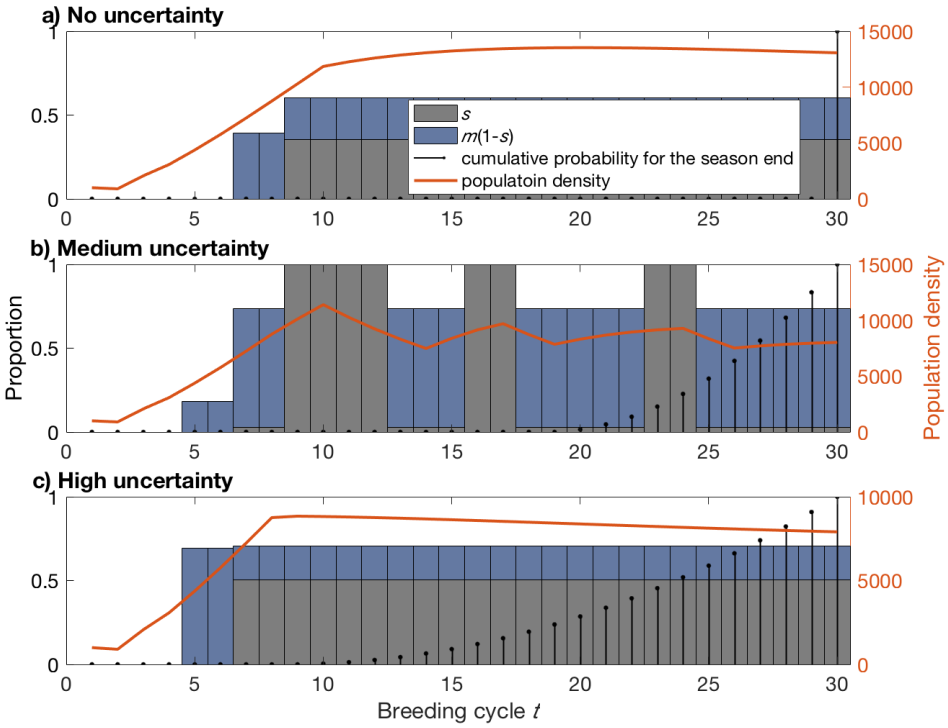

Table S1 Model parameters.

t	Breeding cycle within the season
T	Breeding cycle when the season ends reaching from T_{min} to T_{max}
p_T	Probability that the season ends after T breeding cycles
W	Fitness of a clone
G_T	Gene copies that exist in ephippia at the end of the season T
N_t	Population density at time t
c	Individuals that mature from one clutch
s_t	Probability of reproducing sexually at time t
	s_t^r Probability of *resident* females reproducing sexually at time t
	s_t' Probability of *mutant* females reproducing sexually at time t
m_t	Conditional probability of producing males if reproduction was not sexual
	m_t^r probability of *resident* females reproducing sexually at time t
	m_t' probability of *mutant* females reproducing sexually at time t
d_f	Mortality of mature females
d_m	Mortality of mature males
F_t	Number of mature females at breeding cycle t
	F_t^r Number of resident females at breeding cycle t
	F_t' Number of mutant females at breeding cycle t
M_t	number of mature males at breeding cycle t
	M_t^r Number of resident males at breeding cycle t
	M_t' Number of mutant males at breeding cycle t
g_t'	Fitness gain of the mutant at time t
p	Probability that recombinant strategies can copy the neighbors strategy

CHAPTER III

Daphnia Invest in Sexual Reproduction When Its Relative Costs Are Reduced

Densities are high

Others are inefficient

Sex should satisfy

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Abstract

The timing of sex in facultatively sexual organisms is critical to fitness, due to the differing demographic consequences of sexual vs. asexual reproduction. In addition to the costs of sex itself, an association of sex with the production of dormant life stages also influences the optimal use of sex, especially in environments where resting eggs are essential to survive unfavourable conditions. Here we document population dynamics and the occurrence of sexual reproduction in natural populations of *Daphnia magna* across their growing season. The frequency of sexually reproducing females and males increased with population density and with decreasing asexual clutch sizes. The frequency of sexually reproducing females additionally increased as population growth rates decreased. Consistent with population dynamic models showing that the opportunity cost of sexual reproduction (foregoing contribution to current population growth) diminishes as populations approach carrying capacity, we found that investment in sexual reproduction was highest when asexual population growth was low or negative. Our results support the idea that the timing of sex is linked with periods when the relative cost of sex is reduced due to low potential asexual growth at high population densities. Thus, a combination of ecological and demographic factors select on the optimal timing of sexual reproduction, allowing *D. magna* to balance the necessity of sex against its costs.

Introduction

Many treatments of the evolution of sex consider sexual and asexual forms as discrete lineages (reviewed in (Lively 2010, Hartfield and Keightley 2012)). In nature, however, competition between these types is often more subtle. Organisms that use sex facultatively potentially gain the 'best of both worlds', as they avoid paying costs of sexual reproduction much of the time, while maintaining access to the benefits of genetic recombination (Green and Noaks 1995, Hurst and Peck 1996, Dacks and Roger 1999). This flexibility brings about a suite of life history consequences: the ability to reproduce either sexually or asexually, depending on current conditions, means that the frequency and timing of the sexual life-cycle is an evolvable trait (Stelzer 2016).

The timing of sex in facultative sexual organisms is critical to fitness for several reasons stemming from the differing demographic consequences of sexual *vs*. asexual reproduction. Asexual reproduction is usually the more efficient strategy in terms of converting resources into offspring, as it avoids the 'twofold cost' of male production (Maynard Smith 1978, Lehtonen et al. 2012). A genotype's asexual and sexual success are not independent, and strongly traded off against each other, because a female can only do one at a time. Switching from asex to sex too early also entails potentially large opportunity costs: foregoing the opportunity to contribute to asexual generations (i.e. current population growth) can, in an exponentially growing population, drastically reduce the representation of a clonal genotype in the mating pool, and hence in the sexually produced offspring generation. This opportunity cost (birth rate disadvantage) of sexual reproduction may be particularly pronounced when offspring gained through sex are not equivalent to offspring produced asexually, as often observed in nature. For example, in facultative sexual organisms sexual offspring are often dormant or dispersing life stages (e.g. Cladocera: (Ebert 2005); rotifers(Schröder 2005, Stelzer and Lehtonen 2016); aphids(Simon et al. 2002)) that do not contribute to current local population growth.

In populations that undergo periods of inhospitable conditions, an association between sexual reproduction and dormancy results in potentially complex selection on the timing of sex. Sexually produced, diapausing offspring are vital for the persistence of a lineage across favourable ('growing') seasons, and must be produced before the intervening periods when the habitat is unsuitable. In such a system, the measure of fitness that is expected to be maximized is the total count of sexually produced dormant stages at the end of the favourable season (Taylor and Gabriel 1993, Serra et al. 2008). In contrast, during the growing season, selection in the short term favours asexual reproduction due to its efficiency (Carmona et al. 2009). If individuals can precisely predict the duration of each favourable season, we expect a simple switch from asexual to sexual reproduction towards the end of the growth season (Aparici et al. 1996, Serra and King 1999, Chapter II). However, in unpredictable environments individuals might hedge their bets with regards to reproductive mode, while in more predictable environments the use of environmental cues could allow this plasticity (Halkett et al. 2004). Indeed, in various facultatively sexual species, changes in temperature (Simon et al. 2002), population density or crowding (Larsson 1991, Stelzer and Snell 2003), food quality (Koch et al. 2009), photoperiod (Zhang and Baer 2000) and predation (Hariston and Olds 1984) contribute to the induction of sexual reproduction. All of these cues potentially inform females about seasonal changes and/or deteriorating conditions, under which sex may be advantageous (Walsh 2013). The reproductive response to such cues can be complicated by

the need to produce both males and sexually reproductive females so that they coincide at sexual maturity during the period optimal for the production of sexual offspring (Galimov et al. 2011, Roulin et al. 2015).

The cost of sex is not a fixed parameter, but may depend itself on current conditions. Investment in each reproductive mode is expected to be influenced by the relative costs of sexual and asexual reproduction. For example, mate-finding costs may be higher at low densities, when encounter rates between individuals are low (Snell and Garman 1986, Sprenger et al. 2011). However, this cost may be diminished if individuals can flexibly switch to asexuality should a mate not be found. Similarly, the cost of sex may depend on the current scope for asexual population growth (Williams 1975, Bell 1982, Burt 2000). Assuming that adults are better able than offspring to survive at high densities, when a population approaches carrying capacity the recruitment rate declines.

A handful of population dynamic models highlight the consequences of these dynamics for the demographic advantage of asexual lineages over sexuals (Doncaster et al. 2000, Lively 2010 and 2011). They show that asexuality cannot always realize its demographic advantage: if high densities prevent immediate population growth, the opportunity cost of sex diminishes for populations nearing carrying capacity. Although this is not always sufficient to modify the cost if sex and asex occur simultaneously (Lehtonen et al. 2012), the prediction for facultative sexuals with dormant sexual eggs is clear: they should switch to sex as resources become limiting and the opportunity cost of sexual reproduction is reduced. This important prediction has, to date, been largely overlooked by empirical studies of the costs and benefits of sex.

In facultative sexual *Daphnia* (Cladocera: Daphniidae) we expect strong selection on the timing of sex. *Daphnia* sex appears costly relative to asexual reproduction in the short term due to its demographic effects. The largest asexual clutches recorded for *D. magna* contain ~110 eggs (Hebert 1978), whereas sexual clutches contain at most two eggs. This clutch size difference represents a potentially extreme opportunity cost of reproducing sexually, which necessarily entails foregoing an asexual reproductive bout. Non-equivalence of sexually and asexually produced offspring holds for this system: asexually produced eggs develop immediately in the maternal brood chamber into free-swimming plankton, whereas fertilized sexual eggs must undergo a period of dormancy, encased in a hardy capsule known as an ephippium (Ebert 2005). Additionally, sexual reproduction requires the (asexual) production of males, which reduces the asexual growth rate of a lineage. However, in terms of resource allocation, the extent to which investment in sex trades off with other life history traits, including asexual investment, is not clear. While production times for sexual and asexual clutches are equal, the large number of eggs in an asexual clutch could translate into higher resource requirements compared to the two eggs per sexual clutch. Alternatively, melanisation of the ephippium and provisioning for dormancy might require additional resources when producing a sexual clutch.

Ultimately, only sexual, dormant eggs are able to withstand harsh conditions, including freezing and desiccation, so sexual reproduction is vital for the long-term persistence of a lineage over inhospitable periods. At the start of each growing season, when environmental conditions become suitable, dormant eggs hatch into females that found the planktonic population anew. Male production and the female switch to sexual reproduction may occur in response to different cues, or with different sensitivity to the same cues: in *D. magna*, pro-

duction of males and of sexual clutches responded differently to manipulations of photoperiod (Roulin et al. 2013), and male production has been observed to occur more stochastically throughout the growing season compared to ephippia production (Galimov et al. 2011). The relative roles of environment and genotype in determining the likelihood of male and ephippia production also vary: for example, in *Daphnia pulex* inhabiting temporary ponds over a short growing season, substantial male production occurred very early while population densities were still low, and was also not linked to other environmental factors such as pond temperature (Innes 1997).

Most studies on the timing of sex and male production in *Daphnia* have focused on cues that allow dormant eggs to be produced before environmental deterioration or the end of the season, and have been conducted under laboratory conditions (e.g. (Zhang and Baer 2000, Roulin et al. 2013 and 2015,Spaak and Boersma 2001 Gyllström and Hanson 2004), but see (Innes 1997)). We aim to add the costs and consequences of sex itself to this picture, and focus on population density as a variable connecting the ecological and demographic influences on the timing of sex. We highlight the hypothesis of demographically varying costs of sex (Doncaster et al. 2000, Lively 2010 and 2011) as an important alternative to the prevailing emphasis on sex as a response to deteriorating conditions. This prevailing view sees sex either providing a direct escape route (e.g. dormancy (Roulin et al. 2013 and 2015)) or generating diversified offspring through recombination, to explain why particularly stressful conditions induce sex (Hadany and Otto 2007).

Of these three options, we focus on the first two (the demographic cost hypothesis, and the habitat deterioration hypothesis). The third hypothesis appears unlikely to explain the precise scheduling of sex in the current context. While high density (and its correlates, e.g. increased resource limitation or disease risk) may constitute a stressful environment, it is difficult to envisage a benefit of producing diverse offspring genotypes in response to this transient stress. Offspring hatch in subsequent seasons under benign density conditions; the range of densities a lineage may later encounter is independent of the density when the lineage-founding ephippia were produced.

Returning to the two focal hypotheses, previous work has shown that crowding promotes sex induction and reduces asexual fecundity in laboratory populations of *Daphnia* (Fitzsimmons and Innes 2006). Observing these patterns in natural populations would support the habitat deterioration hypothesis, with support strengthening if populations do not persist after reaching high density. The demographic cost hypothesis, in contrast, predicts that density directly modifies the relative costs of sex and asexual reproduction through its relationship with the population's capacity for growth (Doncaster et al. 2000, Lively 2010 and 2011). In this case, we would expect sex induction to be related to population growth rates in addition to density.

We used an intensive longitudinal sampling regime to document population dynamics and the occurrence of sexual reproduction over the main part of the growing season in natural populations of cyclically parthenogenetic *Daphnia magna*. We investigated the interacting effects of population density, asexual reproductive investment, and growth rates on the frequency of sexually reproducing individuals. Additionally, in the laboratory we estimated resource allocation trade-offs between the production of ephippia and asexual fecundity over the lifespan of individual females, to clarify whether investment in sex imposes costs beyond its immediate demographic disadvantage.

Methods

Population sampling

We sampled 11 natural *D. magna* populations every three-to-four days for 60 days (May 30 – July 28, 2015). Populations inhabited separate rock pools distributed over 6 islands (FU1, HA, K, LON, N, and SMF) in the Finnish archipelago near Tvärminne Zoological Station (59.8420° N, 23.2018° E). We recorded density and demographic structure ('stage-structure') of the populations at each sampling point. To assess population density, 350 ml water samples were collected at 15 haphazardly chosen locations spanning the pool area and depth. These were combined in a bucket and stirred to distribute individuals evenly, and a 350-ml subsample was taken as the final density sample. The remaining animals were returned to the rock pool. After collecting the density sample a small hand net was swept through the pond to take a representative population sample.

Live samples were brought back to the lab and analysed the same day. All *D. magna* individuals in the 350-ml density sample were counted under a dissecting microscope and converted to an estimate of individuals/L. The stage-structure samples were variable in size; to make larger samples manageable (< 1000 individuals) they were split using a Folsom plankton sample divider. The sample was then sieved through 0.6 mm nylon mesh to separate the smallest individuals. Individuals that remained in the sieve were counted and classified into the following categories under a dissecting microscope: females with asexual eggs or embryos in the brood pouch, females with an empty brood pouch but filled ovaries, females with ephippia, adult females without eggs, embryos or filled ovaries, juvenile females (indicated by short 1st abdominal process (Ebert 2005)); adult males (prolonged first antenna, copulatory hook on the first thoracic leg (Ebert 2005, Dodson and Frey 2001)) and juvenile males. After assessing stage-structure, up to ten females (where possible; median = 10, mean ± SE = 8.72 ± 0.17) with asexual eggs in the brood pouch were isolated from the sample and maintained in individual 35 ml jars until they released their clutch. The number and sex of offspring was determined under a dissecting microscope. This paper's focus is the timing of investment in sexual reproduction, and does not present the data on offspring sex allocation, which is addressed in a second study using the population density and stage-structure data collected here (ChapterIV).

Reproductive life history trade-offs

We collected large population samples from five additional rock pool populations and isolated 60 females (F0) carrying asexual clutches. Females were kept individually in 50 ml falcon tubes filled with artificial *Daphnia* medium (ADaM (Klüttgen et al. 1994)) and fed daily with *Scenedesmus* algae (~5 million cells per individual per day) until they released their first clutch. We isolated four F1 daughters per F0 female and housed them in pairs in 50 ml falcon tubes until they produced their first clutch. Twenty-two of these groups of four sister F1 females (henceforth 'clones') synchronously produced enough daughters that we could isolate ten F2 females per clone, half of which were assigned to a long day length treatment (18:6 hours light:dark) and the other half to a short day length treatment (6:18 hours light:dark) on the day of their release from the maternal brood pouch. We used extreme day lengths (naturally occurring at midsummer and midwinter at the study site) to induce propensities for sex that were as different as possible between treatment groups. Over the 35-day exper-

imental period, females experienced these photoperiod treatments under otherwise standardized conditions in climate chambers (20°C, with *Daphnia* placed ~20 cm below the fluorescent light source). Individual F2 females were fed and checked daily for the release of asexual clutches or sexually produced ephippia. When an asexual clutch was released, the date, number and sex of offspring were recorded, the offspring removed and the water changed. When an ephippium was produced, the date was recorded, the ephippium removed and the water changed. We recorded the date of any deaths. Females that did not reproduce were excluded from the analysis. We also excluded 7 females that produced an ephippium in the very first clutch, which needed twice the time to produce their first clutch compared to other females, indicating very unusual behaviour.

Statistical analysis

We were interested in how the frequency of sex relates to population density, population growth, and asexual reproductive effort. We ran separate models to predict the frequencies of sexual females and males, as they could respond differently to these predictors. Models for the frequency of sexual females in a sample used population density from the previous sampling point ('lag density') as a predictor, because these prior conditions (3-4 days before) coincide with the point at which female reproductive mode would have been determined [9]. However, models for the frequency of adult males used current density, as conditions at the previous sampling point do not coincide with the production of these males. The appropriate lag period (the amount of time males need to mature) is at least 10 days/3 sampling points, and a predictor variable using this lag would have unacceptably reduced our sample size. Using current density in the analysis instead allows us to examine whether males are produced so as to coincide with periods of high density in adulthood. As density varied by orders of magnitude across populations, and within populations over time, we used log-transformed density in all analyses. We calculated the intrinsic rate of per capita population growth per time step as $r = \frac{\ln(N_{t2}) - \ln(N_{t1})}{t2 - t1}$ (Begon et al. 1990).

Asexual reproductive effort was estimated from the clutch size of ~10 females, carrying asexual eggs, per population sample. The clutch size among sampled females overestimates the mean population asexual reproductive effort, as a small but variable proportion of mature females in each population sample showed no current reproductive investment (with neither eggs in the brood pouch nor filled ovaries). We weighted the mean clutch size of sampled females by the proportion of currently reproductive females among all mature, non-sexually reproducing females to estimate the mean asexual reproductive effort in the population. We used log-transformed clutch size and reproductive effort in our analyses to normalize their distribution. Because asexual reproductive effort and growth are related (collinearity) we included them separately in models with density as the only other predictor. To determine the threshold asexual reproductive effort at which populations switch to sexual reproduction, we created a binary dependent variable for whether a population sample contained females investing in sexual reproduction or not. We fitted a logistic regression of this variable over asexual reproductive effort and determined its inflection point.

Statistical analyses were performed in R (v. 3.2.2) (RCore Team 2016). We used linear mixed-effects models in the package lme4 (Bates et al. 2015) for analyses of density, clutch size, and growth rates in the natural populations, and of asexual clutch size and mean interval between clutches in the laboratory experiment. For analyses of proportions of males and

sexual females we used generalized linear mixed-effects models (GLMMs) with binomial error and logit link in lme4. To account for repeated measurements the population ID (natural populations) or family ID (laboratory experiment) was included as random factor. If binomial models were overdispersed an observation-level random factor was included (Harrison 2014). Predictor variables in binomial GLMMs were standardized to aid in interpretation of parameter estimates, reported on the log odds scale; as an indication of effect sizes, we also present the odds ratio for each parameter, and marginal and conditional R^2 (Nakagawa and Schielzeth 2013) for each GLMM. Summary statistics are presented as mean ± 1 standard error (SE), unless otherwise specified.

Results

Sex is associated with high density and low asexual reproductive effort

The frequency of sexual females (adult females carrying sexual eggs) was larger following high population densities, and when mean reproductive effort among asexual females was low (Fig. 1, Table 1). The value for asexual reproductive effort at which the majority of populations contained females investing in sexual eggs was 11.47 offspring (Figure S1). The frequency of sexual females also increased with decreasing growth rate *r* (Fig. 1, Table 1). The frequency of males in the adult population was larger when density was high, and when asexual effort was low, but was not significantly related to population growth rate (Fig. 1, Table 1). Odds ratios in Table 1 show the predicted change in odds with each standard deviation increase in the predictor, for a constant (mean) value of the covariate. For example, the odds of a female carrying an ephippium were 0.035 at the intercept (i.e. for mean values of density and asexual effort). For each standard deviation increase in density, keeping asexual effort constant, these odds increased by a factor of 4.75.

The association of higher frequencies of sexual females with high population density and low growth rates can also be seen when looking at patterns across the growing season (Fig. S2). Across all populations, there were two main peaks in density during our sampling period. These peaks are followed by periods of reduced growth rate (Fig. S2) reflecting the negative relationship between population growth and population density (LMM: Slope = -0.053 ± 0.014, χ^2 = 13.83, p <0.001). Lag population density was also negatively related to asexual clutch size (LMM: Slope = -0.26 ± 0.047, χ^2 = 31.02, p < 0.001) (Fig. S2).

Ephippia production trades off with asexual clutch size

The number of females that reproduced in the long-day and short-day experimental treatments did not differ (83 of 110 and 88 of 110, respectively; z = 0.81, p = 0.42). The short-day treatment successfully induced ephippia production, with 41 of 88 females producing at least one ephippium, compared to 1 of 83 females in the long-day treatment (GLMM: Slope = -4.700 ± 1.199, z = -3.92, p < 0.001; Fig. 2). Total asexual reproductive success (number of offspring) was higher in the long-day treatment (LMM: Slope = 7.507 ± 2.475, χ^2 = 9.20, p = 0.002; Fig. 2). Day length did not affect the total number of reproductive bouts, the latency to first reproduction, or the mean interval between clutches (all p > 0.05). Nor did day length affect the mean asexual clutch size when calculated across all of a female's asexual clutches (LMM: Slope = 0.178 ± 0.420, χ^2 = 0.18, p = 0.67). Within the short-day treatment, we could

compare individuals that reproduced only asexually with those that produced at least one sexual clutch. The latter had fewer total asexual offspring, losing on average 9 offspring compared to their clone mates that reproduced only asexually (asexual, 54.1 ± 1.9, sexual, 45.4 ± 3.2; LMM: Slope = -9.167 ± 3.856, χ^2 = 5.65, p = 0.02), but whether or not females reproduced sexually did not affect their mean asexual clutch size (-0.974 ± 0.801, χ^2 = 1.48, p = 0.22). The absolute number of sexual clutches (0, 1 or 2) was not related to mean asexual clutch size (-0.523 ± 0.588, χ^2 = 0.79, p = 0.37). However, the proportion of a female's reproductive events that were sexual was negatively related to her mean asexual clutch size (LMM: Slope = -5.958 ± 2.535, χ^2 = 5.52, p = 0.019); if half of a female's clutches were sexual, the mean size of her asexual clutches was reduced by around 3 eggs compared to females producing only asexual clutches (Fig. 2c). Females that produced relatively many ephippia had smaller clutches when they reproduced asexually.

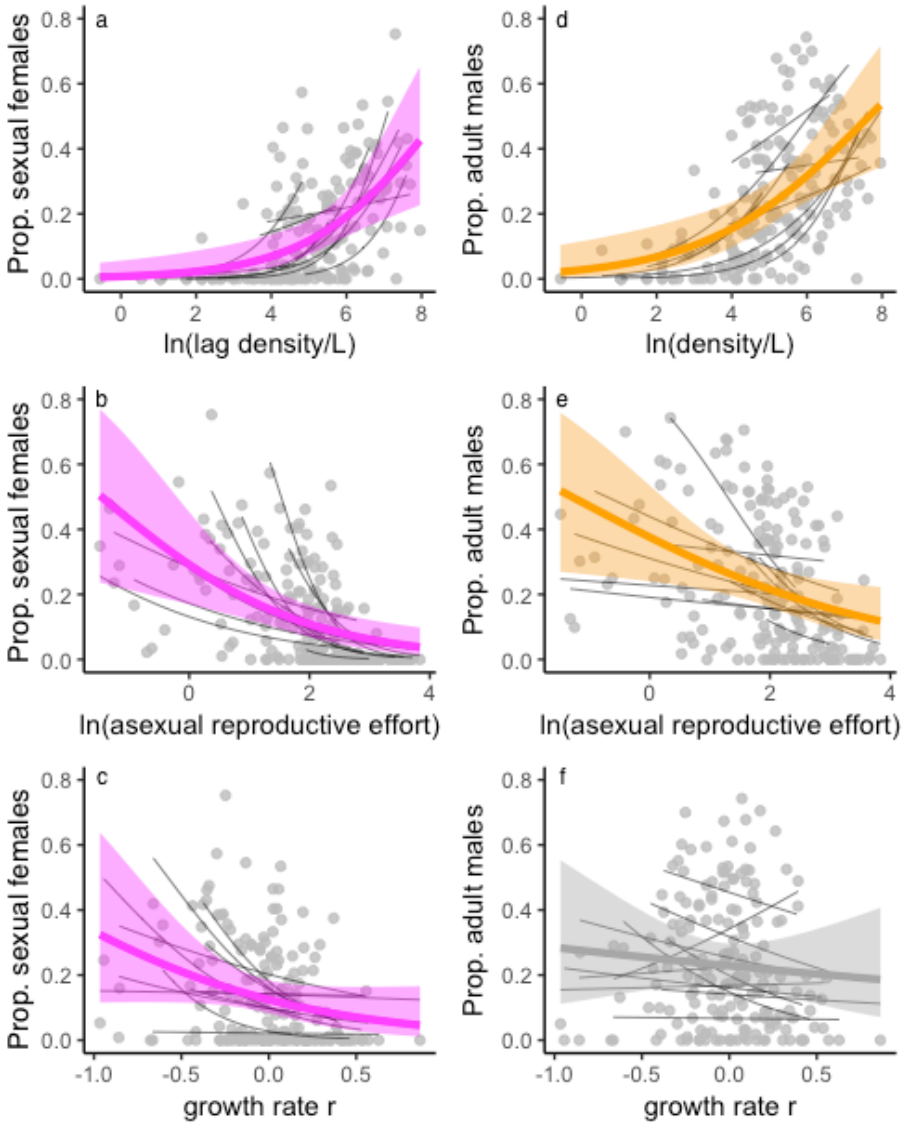

Figure 1. The relationship of the proportion of sexual females (a-c) and males (d-f) with lag (a) or current (d) population density (*Daphnia*/L), asexual reproductive effort (b, e), and growth rate (c, f). Bold lines show the global logistic regression with 95% confidence intervals, with significant relationships in colour and non-significant in grey. Thin black lines show regressions for each population, and light grey points show raw data.

Table 1. Effects of population density, mean asexual reproductive effort (RE), and growth rate on the proportions of sexual females and adult males in a population; estimated by binomial GLMM with logit link. Parameter estimates are presented as the log odds ratio (ß) and its standard error (SE); we additionally present the odds ratio (OR) as a measure of effect size. R2GLMM(m), marginal R2 (variance explained by fixed effects); R2GLMM(c), conditional R2 (variance explained by fixed + random effects).

Model: Density and asexual reproductive effort											
Proportion sexual females (N = 174)						Proportion adult males (N = 185)					
Fixed effects:	ß	SE	z	p	OR	Fixed effects:	ß	SE	z	p	OR
(Intercept)	-3.344	0.333	-10.04	< 0.001	0.035	(Intercept)	-1.878	0.172	-10.91	< 0.001	0.153
ln(lag density)	1.558	0.221	7.04	< 0.001	4.751	ln(density)	0.976	0.129	7.56	< 0.001	2.654
ln(mean asexual RE)	-0.914	0.155	-5.91	< 0.001	0.401	ln(mean asexual RE)	-0.470	0.108	-4.36	< 0.001	0.625
Random effects:	SD					Random effects:	SD				
Population ID	0.983					Population ID	0.466				
Observation ID	1.412					Observation ID	1.206				
R^2:	$R^2_{GLMM(m)}$	$R^2_{GLMM(c)}$				R^2:	$R^2_{GLMM(m)}$	$R^2_{GLMM(c)}$			
	0.426	0.514					0.224	0.258			
Model: Density and growth rate											
Proportion sexual females (N = 166)						Proportion adult males (N = 177)					
Fixed effects:	ß	SE	z	p	OR	Fixed effects:	ß	SE	Z	p	OR
(Intercept)	-3.299	0.274	-12.05	< 0.001	0.037	(Intercept)	-1.910	0.171	-11.15	< 0.001	0.148
ln(lag density)	1.879	0.240	7.83	< 0.001	6.544	ln(density)	1.177	0.144	8.19	< 0.001	3.243
growth rate *r*	-0.389	0.155	-2.52	0.012	0.678	growth rate *r*	0.161	0.114	1.41	0.16	1.175
Random effects:	SD					Random effects:	SD				
Population ID	0.721					Population ID	0.443				
Observation ID	1.586					Observation ID	1.277				
R^2:	$R^2_{GLMM(m)}$	$R^2_{GLMM(c)}$				R^2:	$R^2_{GLMM(m)}$	$R^2_{GLMM(c)}$			
	0.394	0.444					0.191	0.222			

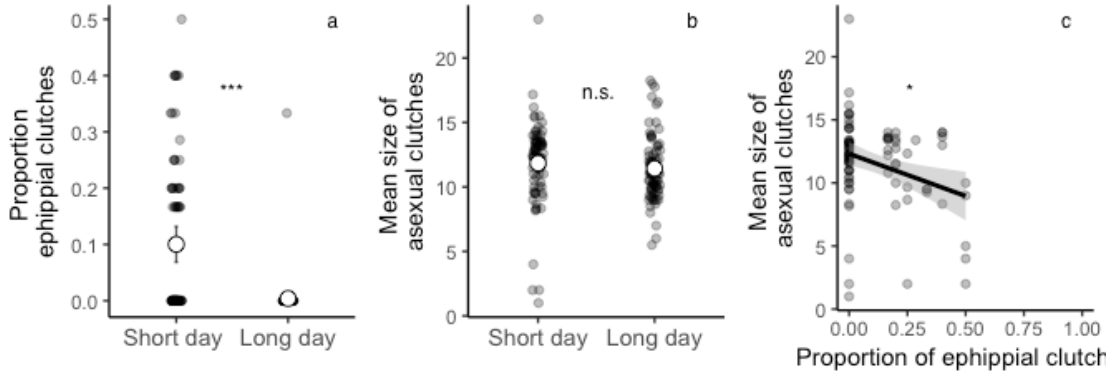

Figure 2 The effect of day length treatment (short-day, 6:18 hours light:dark; long-day, 18:6 hours light:dark) on (a) the mean proportion of a female's clutches that were sexual and (b) the mean size of asexual clutches; and (c) the relationship between the proportion of a female's clutches that were sexual and her mean asexual clutch size, for the short-day treatment only. Asterisks indicate significance (n.s., $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$).

Discussion

Daphnia magna in the rock pool habitat studied here experience a short growing season followed by completely inhospitable conditions in which only dormant, sexually produced eggs persist. Besides winter freezing, pools often experience summer droughts (Altermatt and Ebert 2008). The resulting strong selection for the production of dormant eggs is therefore expected to shape the timing of sexual and asexual reproduction during the growing season. While approaching inhospitable conditions do influence investment in sexual reproduction (Roulin et al. 2013), here we argue that in line with theoretical predictions, the reduced cost of sexual relative to asexual reproduction at high densities may plausibly favour density as a cue for the switch to sexual reproduction in *D. magna*. Our results suggest that an understanding of how population dynamics affect the costs of sex can inform predictions of when sex should occur, and highlight the importance of considering variation in the costs of sex when seeking explanations for the maintenance of sexual reproduction.

Investment in sexual reproduction in our sampled populations was strongly predicted by population density: when density was high at the previous sampling point, a greater proportion of females in the current sample carried sexual eggs. The frequency of males in the adult population also increased with density. These relationships were consistent over the sampled time period, where we observed two density peaks (Fig. S2). However, neither of these peaks in density and sexual reproduction was followed by extinction of the planktonic population, indicating that sex did not immediately precede inhospitable periods where the dormant stage would be vital. This conflicts with the habitat deterioration hypothesis, suggesting that the approach of inhospitable conditions alone cannot explain the marked increase in investment in sex at these points.

We suggest, in accordance with the demographic cost hypothesis, that the association of sexual reproduction and population density results from declining efficiency of asexual reproduction as populations approach carrying capacity — newborn offspring may then have difficulty recruiting into the current population, which reduces the returns on asexual offspring.

Consequently, the relative profitability of sexual reproduction (which does not contribute to current population growth) increases with population density. Furthermore, sexually produced offspring are not affected by current conditions (e.g. competition), as they do not hatch until subsequent growing seasons. We could not measure juvenile survival, which is predicted to decrease with population density under this scenario. However, the frequency of sexual females increased with decreasing (or negative) population growth, and decreasing asexual reproductive effort. Thus, for a given population density, females increased their investment in sexual reproduction when potential growth was low or populations were declining. This supports the demographic cost hypothesis that sex in ephemeral *D. magna* populations is timed to coincide with periods when the opportunity cost of sexual reproduction is reduced (Doncaster et al. 2000, Lively 2010 and 2011) (in addition to the likelihood of sex increasing with ecological cues, such as changes in day length, ensuring that it occurs before the season end (Roulin et al. 2013)). Our data on the clutches of females taken from natural populations suggest that this cost is balanced when the mean asexual reproductive effort in a population is around 11.5 eggs. Below this value, sexually reproducing females could be found in the majority of populations (Fig. S1).

Interestingly, in other facultative sexual systems showing an association between population density and sex induction (e.g. rotifers (Serra et al. 2008,Stelzer and Snell 2003, Stelzer 2012)), density-dependent induction of sex has been shown to contribute to regulating population density in a controlled laboratory setting (Schröder and Gilbert 2004). Because of the production of males and dormant stages, which require resource investment and do not contribute to current population growth, increasing rates of sex can feed back negatively on growth rates (Aparici et al. 1996, Serra and King 1999). In rotifers the density threshold for sex induction is low, and sex is directly related to population density, rather than indirectly through resource depletion (Stelzer and Snell 2003), suggesting that the induction of sex influences the growth potential of the population (Stelzer 2012). This effect on population growth has also been demonstrated in laboratory *Daphnia* populations (McCauley et al. 1999). Such negative feedback is also possible in our dataset, but is much more difficult to detect in natural populations due to the many other uncontrolled variables (e.g. pool volume, algal productivity) likely to affect rates of sex, density, and their relationship.

In contrast to the frequency of sexual females, the frequency of adult males was not related to population growth. High male frequencies coincided with periods of high density and low asexual reproductive effort, but generally male occurrence appears to be timed less precisely than ephippia production. This is perhaps not surprising when considering that males require time (~10 days at 20°C) to mature, which reduces the likelihood that cues available when males are produced will reliably predict population dynamics at their maturity. Furthermore, as male lifespan is substantially longer than the moult cycle over which a female bears an ephippium, male frequency increases cumulatively over time while ephippia frequency reflects much more closely the current conditions. Previous studies of *Daphnia* species in temporary habitats have found similar patterns of male appearance in a population preceding the first production of ephippia (Innes 1997,Galimov et al. 2011).

High population density leads to increased investment in both males and ephippia in laboratory populations of *Daphnia* (Larsson 1991,Kleiven et al. 1992, Berg et al. 2001, Olmstead and LeBlanc 2001) and to smaller asexual clutch sizes (Fitzsimmons and Innes 2006). A negative relationship between density and asexual reproduction was also apparent in our dataset. This could reflect increased competition at high densities, resulting in reduced repro-

ductive condition that restricts female fecundity. However, we observed increased sexual reproduction at high densities, when resources are limited. This finding is difficult to reconcile with the suggestion that the sexual ephippia have a high resource cost (Lynch 1983). If resources limit the production of large asexual clutches (as shown in many experiments, e.g. (Lynch 1989, Guisande and Gliwicz 1992), these conditions should also constrain production of costly ephippia. Our laboratory results on reproductive trade-offs suggest that producing a sexual clutch is costly: individual females producing a greater proportion of sexual clutches over their lifespan produced, on average, smaller asexual clutches. The cost imposed on asexual reproductive potential by a sexual event is thus greater than the loss of one asexual clutch. However, quantifying the absolute cost of producing a sexual clutch requires experiments manipulating asexual clutch sizes by altering resource availability.

The major cost of sex in our experiment appeared to be the immediate trade-off arising from the inability to produce a sexual and asexual clutch simultaneously: females that produced more ephippia had a lower total number of asexual offspring. If a female producing a sexual clutch has fewer opportunities and/or resources left available for asexual reproduction, there are clear consequences for the competitiveness of clonal lineages with different propensities for sexual reproduction in terms of their numerical representation in the population. *D. magna* clones vary in their propensity to produce males and, independently, ephippia in response to environmental cues (Roulin et al. 2013 and 2015). Sexually produced, dormant offspring are the measure of long-term fitness in *Daphnia* and many facultative sexual organisms, but total sexual output depends both on sexual and asexual fecundity. The timing of sexual reproduction is thus expected to optimise investment in the two reproductive modes (Chapter II).

Conclusions

In wild populations of facultative sexual *D. magna,* females invest in sexual reproduction following high population densities and when the population growth rate and asexual reproductive effort are low, conditions that reduce the relative cost of sexual reproduction. We provide empirical support for the idea that a facultative sexual population will show increased rates of sex as it approaches carrying capacity and the cost of sex declines. Combining our new finding with previous results we suggest that three underlying rules determine the induction of sexual reproduction in *D. magna* on a large biogeographic scale: First, ephemeral, seasonal populations that frequently experience inhospitable periods should generally invest more in sexual reproduction compared to populations in permanent, less seasonal habitats (Doncaster et al. 2000). Second, we have found that within a season, sex induction co-occurs with conditions that are theoretically predicted to reduce its costs relative to asexual reproduction (Doncaster et al. 2000, Lively 2010 and 2011). This is the case at high population densities when asexual clutch size is small and the cost of foregoing asexual reproduction is low. Third, previous studies have shown that this pattern can be modified by the timing and predictability of onset of inhospitable conditions, such that investment in sexual reproduction increases towards the anticipated end of the growing season (Roulin et al. 2013, Chapter II). We conclude that timing of sex in cyclical parthenogens is not only shaped by the approach of inhospitable conditions, but appears to respond to effects of density and population growth on the relative costs of sexual and asexual reproduction.

Acknowledgements

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Data accessibility

All data are archived in the DRYAD digital repository (DOI provided on manuscript acceptance)

Authors' contributions

NG, IB, DE and HK conceived the study; NG, IB and DE designed the study; NG and IB carried out data collection, analysed the data, and drafted the manuscript; all authors revised the manuscript and gave final approval for publication.

Appendix Chapter III

Figure S1 Occurrence of sexually reproducing females in the population with respect to asexual reproductive effort. Grey lines show the logistic regression within populations, whereas the pink line shows the logistic regression across populations. The inflection point (black line) indicates the threshold mean asexual reproductive effort (e2.44=11.47) at which the majority of populations contain females that carry a sexual clutch. Light grey dots show the raw data.

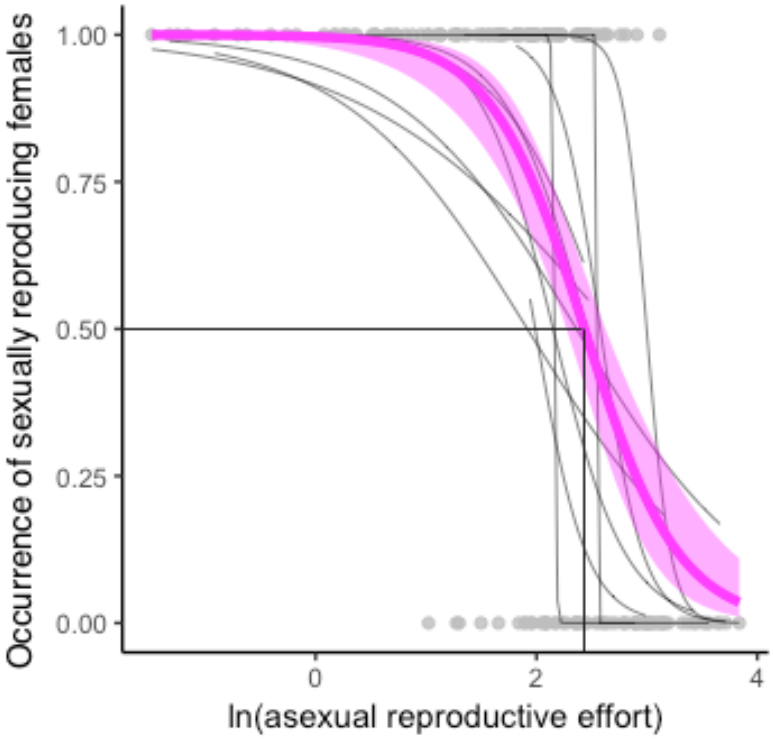
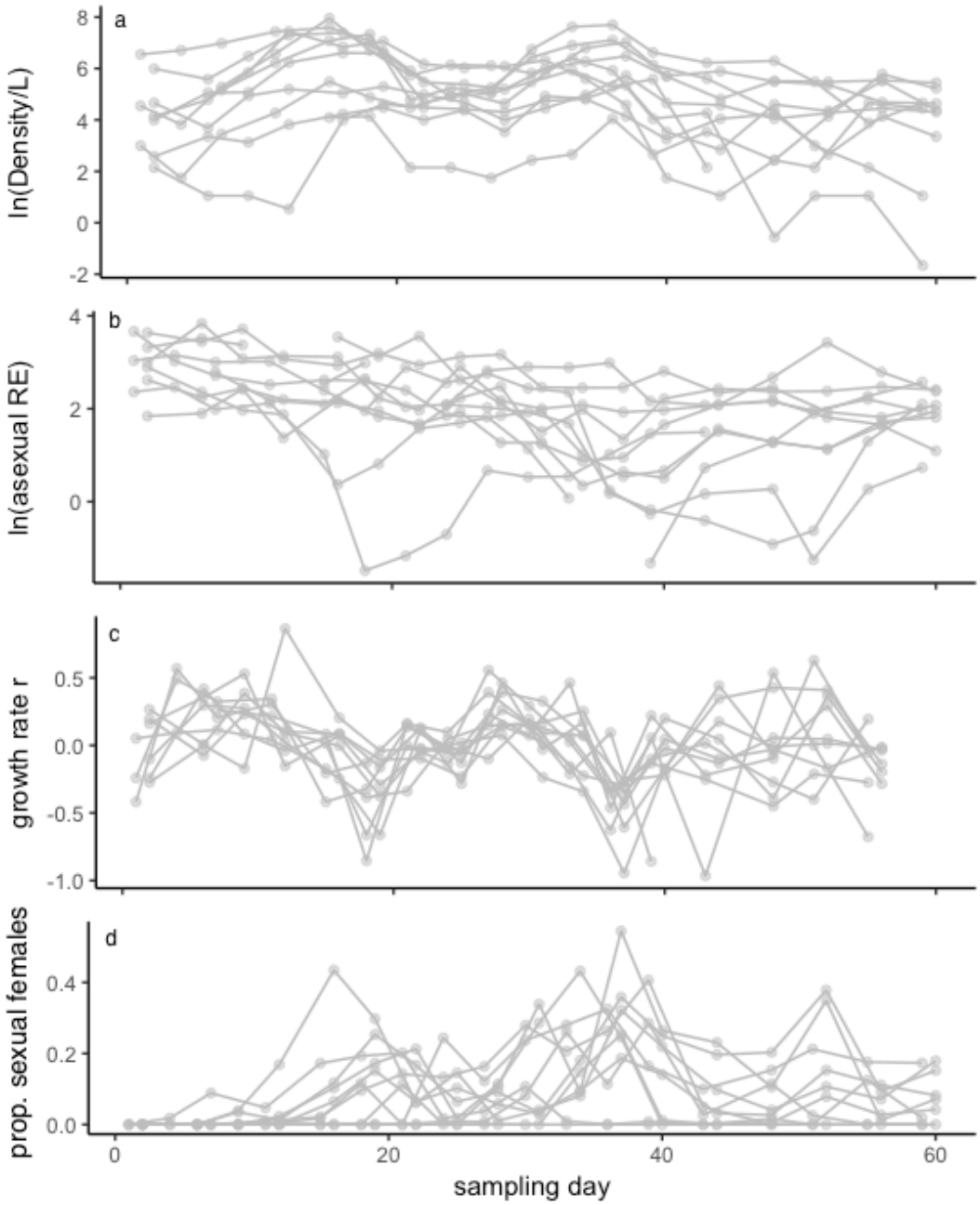


Figure S2 Temporal dynamics (day 1 = May 30, 2015) of (a) population density (Daphnia/L), (b) asexual reproductive effort, (c) population growth rate, and (d) the proportion of sexual females in the population. Each line shows one of the 11 rock pool populations.



CHAPTER IV

Daphnia Females Adjust Sex Allocation in Response to Current Sex Ratio and Density

Sex is in the air

Some females are still alone

A son will do well

Isobel Booksmythe, **Nina Gerber**, Dieter Ebert & Hanna Kokko. *In Revision* for *Ecology Letters.*

Abstract

Cyclical parthenogenesis presents an interesting challenge for the study of sex allocation, as individuals' allocation decisions involve both the choice between sexual and asexual reproduction, and the choice between sons and daughters. Male production is therefore expected to depend on ecological and evolutionary drivers of overall investment in sex, and those influencing male reproductive value during sexual periods. We manipulated experimental populations, and made repeated observations of natural populations over their growing season, to disentangle effects of population density and the timing of sex from effects of adult sex ratio on sex allocation in cyclically parthenogenetic *Daphnia magna*. Male production increased with population density, the major ecological driver of sexual reproduction; however, this response was dampened when the population sex ratio was more male-biased. Thus, in line with sex ratio theory, we show that *D. magna* adjust offspring sex allocation in response to the current population sex ratio.

Introduction

Sex allocation theory was developed to explain the observation, common across diverse taxa, of equal ratios of male to female progeny. Fundamentally, over-production of one sex reduces the expected fitness payoff from individuals of that sex, thereby selecting for increased production of the opposite sex (Fisher 1930; Düsing 1884; Edwards 2000). This principle of negative frequency-dependence has been successfully applied across an impressive range of life histories, breeding and genetic systems (West 2009), and shown to generalize to cases where equilibrium offspring sex ratios (proportion of male offspring) are not 50%, in which case equal investment into production of each sex is predicted (if males and females are not equally costly to produce, more offspring of the cheaper sex are expected; Charnov 1982; but see Kahn *et al.* 2015).

Trivers and Willard (1973; see also Charnov 1982) recognized that, given differential environmental effects on male and female fitness, reproducing individuals would benefit by adjusting their relative investment towards the sex with higher reproductive value. Fluctuations in population sex ratio over time, arising for example through seasonality, can make the reproductive values of sons and daughters differ. Thus, assuming that individuals can measure the current sex ratio or a correlate (e.g. the current season), the primary sex ratio (sex ratio among offspring at production) can evolve to respond to the changing reproductive values (Werren and Charnov 1978, West and Godfray 1997, Kahn *et al.* 2013).

Werren and Charnov's (1978) models were built on specific scenarios of seasonal variation or unusual perturbations to sex-specific fitness expectations. Although the theory lacks extensive further development (West 2009), the idea of facultative sex ratio adjustment in response to population sex ratio remains popular in the empirical literature. Observational data provide mixed support (positive: lions, *Panthera leo*: Creel and Creel 1997; snow skinks, *Niveoscincus macrolepidotus*: Olsson and Shine 2001; northern goshawks, *Accipiter gentilis*: Byholm *et al.* 2002; negative: reed warblers, *Acrocephalus arundinaceus*: Bensch *et al.* 1999; gray-tailed voles, *Microtus canicaudus*: Bond *et al.* 2003). Robust experiments manipulating sex ratio while controlling for other potential cues are rare, and again provide mixed results. Southern green stink bugs, *Nezara viridula* (McLain and Marsh 1990), parasitic mites, *Hemisarcoptes coccophagus* (Izraylevich and Gerson 1996), a perennial herb, *Begonia gracilis* (Lopez and Dominguez 2003), and southern water-skinks, *Eulamprus tympanum* (Robert *et al.* 2003) have been shown to produce male-biased offspring sex ratios when kept in female-biased populations, and *vice versa*. However, equally many experiments have failed to find the expected pattern (guppies, *Poecilia reticulata*: Brown 1982; common lizards, *Lacerta vivipara*: Le Galliard *et al*. 2005; southern water-skinks, *E. tympanum*: Allsop *et al*. 2006; jacky dragons, *Amphibolurus muricatus*: Warner and Shine 2007).

Systems with unusual sex ratio dynamics may be particularly useful. Cyclical parthenogenesis describes a life-cycle where females typically produce daughters asexually, but engage occasionally in (potentially environmentally induced) male production and subsequent sexual reproduction (Bell 1982). If females can reproduce asexually as well as sexually, the conditions for plastic adjustment of male production are in place as male production can in principle range from completely unprofitable (when all females opt for asexuality) to highly profitable (when many or all females are sexual). There is little exploration of optimal sex allocation in organisms engaging in both sexual and asexual reproduction (Aparici *et al.* 1998,

2002 examine a haplodiploid case). Cyclical parthenogenesis has several consequences for sex allocation theory. First, because daughters' reproductive value is not as tightly bound by frequency-dependence as in organisms where every individual has both a mother and father, cyclical parthenogens can show extremely female-biased sex ratios. Second, reproductive decisions in cyclical parthenogens involve not only the sex of offspring, but also whether and when to reproduce sexually. Fitness consequences of these decisions are intertwined: male production (which is often asexual) makes little sense unless there are sexually reproducing females in the population. In addition, sex can entail a range of genetic, demographic, and ecological costs and benefits compared to asexual reproduction (Halkett *et al.* 2006; Paland and Lynch 2006; Auld *et al.* 2016), and sexually-produced young sometimes face a different developmental fate: for example, in *Daphnia*, only sexually-produced offspring undergo dormancy before hatching. Finally, additional complexity arises when only some individuals switch to sex, while others continue asexual reproduction. The co-occurrence of asexually- and sexually-reproducing generations may make it difficult for females to measure the current sex ratio and to use it as a basis of reproductive decision-making when offspring fitness is realized later.

Cyclically parthenogenetic *Daphnia magna* meet theoretical assumptions for facultative adjustment of offspring sex in response to the population sex ratio (Werren and Charnov 1978), exhibiting overlapping generations and temporal sex ratio variation. Female *Daphnia* can produce three kinds of diploid offspring: asexually produced males and females, and sexually produced resting eggs, which require fertilization by males. Several generations fit into one summer growing season, during which individual females reproduce iteroparously, switching back and forth between sexual and asexual reproduction, and between producing male or female asexual clutches. *Daphnia* hatching from resting eggs (in subsequent growing seasons) are invariably female. The sex of asexually (ameiotically) produced offspring is environmentally determined: both males and females are genetically identical to their mothers. *Daphnia* thus allow us to distinguish between sex ratio adjustment, by which we mean the sex ratio among asexual offspring (and which we measure in both natural and experimental settings), and sex allocation, which we use when referring to allocation decisions between the production of males and fertilizable eggs (this we measure in natural populations). Our decision to use 'sex allocation' for the latter allocation decision concurs with the standard use of this term in obligate sexuals with separate sexes. Earlier results have shown male production to start before females begin to switch to the production of sexual eggs — an intuitively expected pattern when males need time to mature before they can fertilize eggs (N. Gerber, I. Booksmythe, H. Kokko, submitted manuscript).

Once males are present in the population, predictions for subsequent sex allocation and sex ratio adjustment become less straightforward, as the option of asexual reproduction means that not all females 'count' in the manner assumed by Fisherian sex ratio theory. Extrapolating from the literature on crowding effects, we expected increased male production with increasing population density. However, if *Daphnia* adjust offspring sex to optimise the reproductive value of offspring, male production should decrease with increasing population sex ratio. This creates an experimental opportunity to manipulate density and sex ratio independently, to see if the sex composition of conspecific density matters in individuals' sex ratio adjustment and sex allocation.

A previous study of *D. magna* ruled out the strict alternation of sexes of consecutive broods on detection of a 'male-inducing' cue, and hypothesised that the observed increase in popula-

tion sex ratio over time was due to individual females adjusting, on a brood-by-brood basis, their responses to their current environment (Barker and Hebert 1986). Although seasonal environmental cues, notably day length, play a role in male production (Stross and Hill 1965; Carvalho and Hughes 1983; Hobaek and Larsson 1990), population density is one of the best-known ecological predictors of male production (Hobaek and Larsson 1990; Kleiven *et al*. 1992, Berg et al. 2001) and sexual reproduction (Carvalho and Hughes 1983).

In this study, we test whether female *D. magna* adjust between the three possible offspring types according to the current population sex ratio. We documented patterns of sex allocation in natural populations over the growing season, and manipulated the density and sex ratio of experimental populations to disentangle the effects of these parameters on female allocation decisions.

Methods

Sex allocation in natural populations

Data on offspring sex ratios of individual females was collected during a study of the timing of sex in natural *Daphnia magna* populations (N. Gerber, H. Kokko, D. Ebert, I. Booksmythe, submitted manuscript). We sampled 11 populations inhabiting separate rock pools distributed over 6 islands in the Finnish archipelago at Tvärminne Zoological Station (59.8420° N, 23.2018° E) over two months during the summer growing season of 2015. Every three to four days we recorded the density and demographic structure of every population. To estimate population density, 350-ml samples were collected at 15 haphazardly chosen locations spanning the pool area and depth. These were combined and stirred to distribute individuals evenly, and a 350-ml subsample was taken as the final density sample. The remaining animals were returned to the rock pool. After collecting the density sample a small hand net was swept through the pond to take a representative population sample. All D. magna individuals in the density sample were counted under a dissecting microscope and converted to an estimate of individuals/l. Population samples were classified into categories of age and reproductive status: juvenile males and females, adult males, and adult (reproductively mature) females, which were further classified as sexually reproducing (carrying an ephippium, the melanised capsule into which the fertilized resting eggs will be deposited) or not (asexually reproducing and non-reproductive). Up to 10 females (where possible; median = 10, mean ± SE = 8.72 ± 0.17) with an asexual clutch visible in the brood pouch were then isolated from the sample and maintained in individual 35 ml jars until they released their clutch. Clutch size and sex of the offspring were determined under a dissecting microscope, and we recorded whether the mother formed an ephippium for her next instar, which is visible by a darkening and change in shape of the female's carapace around the brood pouch.

Experimental populations: manipulation of sex ratio and density

Manipulations of population sex ratio and density were carried out in three separate experiments, in July 2015, June 2016 and July 2016. In July 2015, stocks of twenty *D. magna* clones that had been previously collected from the study population, treated with antibiotics to clear microsporidian infections, and maintained in the lab for a year (see Roulin *et al.* 2015) were established in 9L buckets (one clone per bucket; 10-20 founding individuals per clone) outside, near to natural rock pools containing *Daphnia*, so they were exposed to the natural

climate and weather conditions. Buckets were filled with 8L water from a rock pool not used in our observational study, in which no *Daphnia* were detected during the study period. The water was filtered through 48-µm mesh to avoid possible contamination with *Daphnia*, other large plankton or predators. Algae small enough to pass through the filter were allowed to grow and provided a food source for the populations. Each bucket was additionally inoculated with 20 mL of algae suspension (50 million *Scenedesmus* cells/mL) and left to stand for several days before *Daphnia* were added. Filtered water from the same source pool was added to all buckets on two occasions during stock growth to compensate for evaporation.

When stock populations were in the exponential growth phase, pre-reproductive females and males were collected separately and used to create a mixed-clone stock of each sex. While we attempted to include similar numbers of individuals from each clone, availability of individuals varied due to population size differences among the stocks, and the exact representation of each clonal genotype in the stock mixture is unknown. Using individuals haphazardly sampled from these stocks we set up two sets of experimental populations. The first set manipulated sex ratio across four treatment levels, from 0 to 74% male, while holding density constant at 50 individuals. The second set manipulated sex ratio and density simultaneously by adding 0, 10, 25 or 50 males to populations of 50 females (see Table S1, 'Exp 1', in Supporting Information). In June 2016, we repeated this experiment using pre-reproductive *D. magna* females and males collected directly from several natural rock pool populations at the study site; the clonal composition of 2016 stocks was therefore completely unknown. The numbers of females and males used in each treatment level differed slightly from the 2015 experiment, as we included a wider range of density treatments (from 25-100 total individuals, see Table S1, 'Exp 2'). To ensure our treatments had enough replication to comprehensively cover the range of population densities we had used, in July 2016 we set up additional replicated populations in a third experiment, again using animals collected from several natural rock pool populations at the study site, to manipulate the sex ratio (across four levels from 0 to 75% male) while holding density constant at a low (25 individuals) or high (100 individuals) level (see Table S1, 'Exp 3'). Experimental populations in both years were established in 9L buckets containing 8L filtered water from the same source used for the stock populations, each inoculated with 20 mL of *Scenedesmus* (50 million cells/mL) and left to stand for several days before adding *Daphnia*.

Populations were monitored for maturation of the founding females and the presence of neonates. After two weeks, roughly two juvenile cohorts were apparent in the populations (two size classes of neonates). Each entire population was collected and the number and sex of juveniles determined.

Statistical analysis

Summary statistics are presented as mean ± 1 standard error (SE), unless otherwise specified. The relationship of clutch size with population density was tested in a linear mixed model (LMM) including population as a random factor. To analyse offspring sex ratio and sex allocation data from natural populations we used generalized linear mixed-effects models (GLMMs) with binomial error and logit link in the R package lme4 (Bates *et al*. 2015). Population density, adult sex ratio, and clutch size were included as fixed-effect covariates; the natural log of density and clutch size were used to normalize these variables. To account for repeated measurements population was included as a random factor. If binomial models

were overdispersed an individual-level random factor was included (Harrison 2014). We initially included all two-way interactions between predictors, and sequentially excluded non-significant interactions to obtain final models.

Analyses of the experimental populations were performed in MATLAB. We compared a set of candidate logistic regression models predicting offspring sex, based on model AIC scores. In addition to a 'null' model (intercept-only; neither density nor sex ratio was allowed to predict the proportion of males produced) we built models in which the total density of founders was included as a predictor, and models in which the densities of male and female founders were included as separate predictors that could independently affect the production of males. In each case, we also considered a model variant where estimates from the three experiments were allowed to vary in their effects.

Results

Sex allocation in natural populations

The mean clutch size among asexually reproducing *D. magna* females sampled from natural rock pool populations was 11.97 ± 0.26 offspring (N = 1614; range: 1 – 116 offspring). Clutch sizes were significantly smaller at higher population densities (LMM: –0.084 ± 0.011, X^2 = 57.24, p < 0.001). Asexual clutches are predominantly single-sex (Barker and Hebert 1986; 91.6% of clutches in our data) and mixed-sex clutches are usually strongly biased towards one sex. For the following analyses, we present results in which clutches were assigned their majority sex, ignoring any sons produced in majority female clutches. However, results of all analyses were qualitatively extremely similar (no changes of sign or significance) if we used 'at least one male' as the criterion for male production.

Fixed effects:	ß	SE	z	p
(Intercept)	-3.821	0.423	-9.04	< 0.001
Log density	0.247	0.061	4.03	< 0.001
ASR	0.879	0.413	2.13	0.033
Log clutch size	0.467	0.099	4.70	< 0.001
Random effects:	SD			
Population ID	0.474			
Individual ID	0.00002			

Table 1. Effects of log population density, adult sex ratio (ASR) and log clutch size on the likelihood an asexual clutch was male-biased (N = 1614) in females collected from natural populations; estimated by binomial GLMM with logit link.

As population densities increased, the adult sex ratio (ASR, adult males:adult females) became more male-biased (GLMM: 0.648 ± 0.078, z = 8.33, p < 0.001). The overall mean probability that an asexual female produced a male-biased clutch was 0.22 ± 0.01. Clutches were more likely to be male-biased when sampled from higher-density populations, when the clutch was larger, and when the ASR was more male-biased (Table 1, Fig. 1). The likelihood that a female that released an asexual clutch subsequently produced an ephippium (0.14 ± 0.01) increased when the ASR was more male-biased (Fig. 1), when the female's clutch was male-biased (Table 2), and when the female's clutch was small in absolute terms (Table 2).

However, the size of a female's asexual clutch relative to others in the same population sample had a positive effect on ephippia production: females that produced relatively large clutches were more likely to subsequently produce an ephippium. When accounting for these effects, the effect of density on ephippia production was not significant (Table 2), but excluding them for comparison with previous studies, the effect of density alone was significantly positive (GLMM: 0.740 ± 0.091, $z = 8.09$, $p < 0.001$).

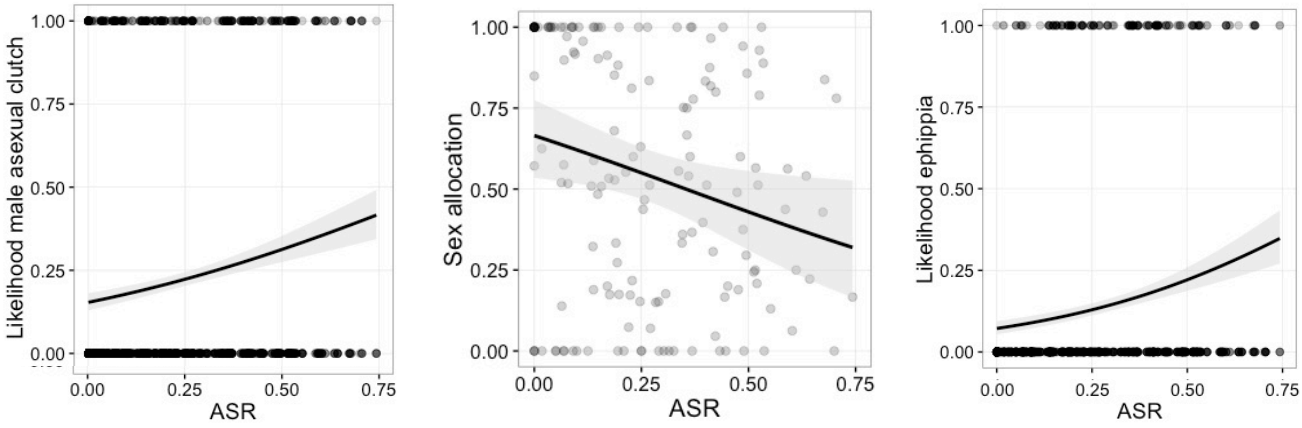


Figure 1. Relationships between the adult sex ratio (ASR) of natural populations and the likelihood a sampled female's asexual clutch was predominantly male (top), the likelihood a female produced an ephippium (middle), and the sex allocation ratio in the population (production of male-biased clutches relative to ephippia, bottom). Darker shading indicates higher density of overlapping raw data points.

We also considered sex allocation in terms of the ratio of investment into male function (producing sons asexually) *vs.* sexual female function (switching to sexual reproduction, i.e. producing ephippia). Adult males and ephippial females are the 'sexual individuals' that make up the mating pool. Note that allocation towards sexual female function is not the production of daughters through ephippia, but the likelihood of ephippia production – that is, the likelihood that the female herself switches to sexual reproduction. To obtain this ratio, we treat a clutch (asexual or ephippial) as the 'unit' of offspring. We know from our population samples the proportion of currently-reproducing females carrying ephippia (0.17 ± 0.02) and the proportion carrying asexual clutches (0.83 ± 0.02). Multiplying the proportion of asexually-reproducing females by the probability that an asexual clutch was predominantly male or female (0.22 ± 0.01 or 0.78 ± 0.01, respectively), we obtain estimates of relative allocation towards clutches of ephippia, sons, and daughters (e.g. using mean values, ephippia:sons:daughters = 0.17 : 0.18 : 0.65). We can use these proportions to approximate relative allocation among sexual functions, i.e. sons:ephippia (e.g. 0.18 : 0.17 = sex allocation ratio of 0.51). Defined this way, sex allocation decreased at higher population densities (Table 3) and when the current ASR was male-biased (Table 3, Fig. 1).

Table 2. Effects of log population density, adult sex ratio (ASR), previous clutch size and sex, and relative previous clutch size on the likelihood a female produced an ephippium (N = 1356) in females collected from natural populations; estimated by binomial GLMM with logit link.

Likelihood of individual female ephippia production				
Fixed effects:	ß	SE	z	p
(Intercept)	-1.685	1.397	-1.21	0.228
Log density	0.106	0.236	0.45	0.656
ASR	1.436	0.554	2.59	0.010
Log asexual clutch size	-2.235	0.685	-3.26	0.001
Relative clutch size *	0.625	0.290	2.16	0.031
Asexual clutch sex (0=female, 1=male)	0.809	0.188	4.31	< 0.001
Log density × log previous clutch size	0.289	0.114	2.54	0.011
Log previous clutch size × relative clutch size	-0.278	0.131	-2.12	0.034
Random effects:	SD			
Population ID	0.694			

*** Relative clutch size** is the clutch size standardized within a sample, i.e. relative to the mean clutch size for females collected from the same population at the same sampling point.

Table 3. Effects of log population density and adult sex ratio (ASR) on the production of male clutches relative to ephippia (N = 185) in natural populations; estimated by binomial GLMM with logit link.

Fixed effects:	ß	SE	z	p
(Intercept)	5.952	1.343	4.43	< 0.001
Log density	-0.831	0.272	-3.06	0.002
ASR	-3.432	1.747	-1.97	0.049
Random effects:	SD			
Population ID	0.593			
Individual ID	3.102			

Sex ratio adjustment in experimental populations

No experimental population had produced any ephippia by the end of the experiment, so our tests refer to sex ratio adjustment among asexually produced offspring. We quantified the proportion of sons among all offspring produced after two weeks (~2 clutches). Model selection based on AIC scores identified an unambiguous best model: that in which male and female density independently affected the proportion of sons produced, and in which their effects were allowed to vary between the three experiments (Table 4). Despite the best model estimating the effects of male and female density separately for each experiment, these effects showed a remarkably consistent pattern across the three experiments. Increasing female density always predicted a greater increase in the likelihood of producing sons than did increasing male density (Fig. 2: isoclines are steeper when female than when male density changes). In experiment 1, adding one male was equivalent to adding 0.51 females in terms of the sex allocation response; in experiments 2 and 3, the corresponding numbers are 0.37 and 0.45.

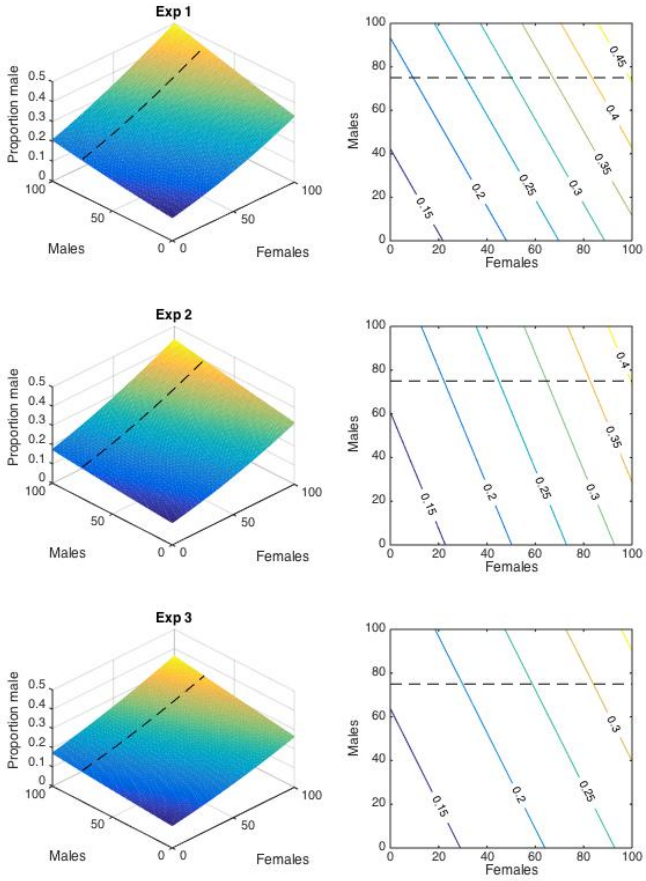


Figure 2. Effect of increasing male and female density on the proportion of male offspring produced in the experimental populations. Although our experiments did not include populations with more than 75 males, predictions for these cases (above the dashed lines) are included to ease visual comparison of male and female slopes.

Table 4. Comparison of candidate models predicting the likelihood of producing males in populations manipulating the density and sex ratio of founding adults.

Model predictors	AIC *	Δ_i †	Estimated parameters ‡
Female density, male density experiments separate	45521	0	$-2.023 + 0.013*D_{f1} + 0.007*D_{m1} + 0.013*D_{f2} + 0.005*D_{m2} + 0.010*D_{f3} + 0.005*D_{m3}$
Female density, male density experiments combined	45542	21	$-1.968 + 0.012*D_{f} + 0.006*D_{m}$
Total density experiments separate	45562	41	$-1.858 + 0.009*D_{(f+m)1} + 0.006*D_{(f+m)2} + 0.006*D_{(f+m)3}$
Total density experiments combined	45591	70	$-1.833 + 0.008*D_{(f+m)}$
Null (intercept only) experiments separate	45724	203	$-1.197 + D_{(f+m)1} + D_{(f+m)2} + D_{(f+m)3}$
Null (intercept only) experiments combined	45774	253	$-1.197 + D_{(f+m)}$

* AIC, Akaike Information Criterion.

† Δ_i, difference in AIC score from the lowest score.

‡ D, density; subscripts *f* and *m* indicate female and male densities, respectively; subscript numerals indicate the experimental block.

Discussion

While much of sex allocation theory enjoys good empirical support, evidence of primary sex ratios responding to the current sex ratio in natural populations remains relatively scant (see West 2009, chapter 8). Our study is conducted in a system where sex itself is facultative, creating unusually strong opportunities for sex allocation responses: male production yields little fitness benefit during periods when most females are not reproducing sexually. Sex ratio adjustment conceivably extends to allow females to respond to the current ASR, which also influences the success of any sons produced. However, if females use conspecific density as a cue for sex (and not only seasonal cues such as day length, e.g. Roulin *et al*. 2013, 2015), it is not straightforward to predict how females should respond to an increased density of females or males. Female abundance does not reliably indicate future mating opportunities for males (as these females might simply keep reproducing asexually), while male presence is a double-edged sword. On the one hand, it can indicate that the population has already partially transitioned to sexual reproduction, and females can gain fitness by producing males who then fertilize sexual eggs. On the other hand, a high ASR also means that any males produced will experience high competition, lowering their expected reproductive success. The former effect predicts that male presence could trigger females to produce more males, the latter argues for inhibition.

In natural populations, the former effect is supported at first sight, as females from more male-biased populations were more likely to produce sons than were females from female-biased populations (Table 1, Fig. 1). Our experiments showed that females respond to male presence in a remarkably consistent manner: if density was increased by adding males, production of sons increased, but only by 37% – 51% of the increase observed if the additional density consisted of females. This suggests that both factors above play a role: females produced sons when densities were high, with a dampened response if the ASR was already high.

In the sampled natural populations, the likelihood of ephippia production – the other aspect of sexual reproduction — increased with the ASR. This is not a response to mating, as *Daphnia* females commit to ephippia production several days before mating. The results match our expectations that investment in sexual female function increases when males are more common. This effect is not solely due to both ephippia and male production increasing independently in response to the same conditions promoting sexual reproduction. The negative effect of male presence on production of sons, and its positive effect on ephippia production, was clearly evident when considering female investment in sexual function only (i.e. sex allocation). Females from more male-biased populations were less likely to produce males relative to ephippia than were females from female-biased populations (Table 3, Fig. 1).

The argument from sex ratio theory that increasing male-male competition reduces the expected reproductive value per son (Frank 1990) provides a logical reason why male abundance inhibits male production, when measured relative to the effect caused by female abundance. We also found greater responsiveness in ephippia production (compared with male production) to current population sex ratio, which makes sense when considering that males cannot mature instantly. Delays between male production and maturation can also help understand why responses to female abundance can be adaptive without the responding individual being necessarily able to measure the competitor female's current life-cycle

status (sexual or not): all else being equal, more females mean more fertilization opportunities in the future, given that each female is able to switch back and forth between reproductive modes.

The monogonont rotifer genus *Brachionus*, haplodiploids in which sexually- and asexually-reproducing individuals are produced in distinct generations, provides the only other studies of sex allocation in cyclical parthenogens (Aparici *et al.* 2002). Here the trait underlying sex allocation is the threshold age at which females lose the capacity to be fertilized. Females that reach this age unfertilized produce only males, thus the earlier the fertilization threshold, the more likely is a female's lifetime sex allocation to be male-biased (Aparici et al. 1998). While there is no such direct effect of male frequency on Daphnia sex ratio adjustment, there are similarities: increasing densities induce sexual generations in Brachionus (Serra and Snell 2009) and production of males and ephippia in Daphnia (Carvalho and Hughes 1983).

Sex ratios also covary with density in species with strong local mate competition (LMC). In fig wasps, females on low-density patches with few founders produce only enough sons to fertilise their daughters, while females at high density produce higher offspring sex ratios (e.g. Herre 1985, 1987). However, LMC seems unlikely to explain the sex–density link in *Daphnia*. Population density does not reflect *Daphnia* clonal diversity, especially several generations into the season, making it an unlikely proxy for LMC. Additionally, an earlier experiment in the same *D. magna* population (Altermatt and Ebert 2008) found no relationship between the initial size of experimental populations (i.e. number of founders) and the total production of ephippia over 100 days of the summer growing season.

Our results agree with the expectation that ephippia production should not begin before male production. This helps explain the remaining conflict between our observational and experimental results: if adding females leads to a stronger male-producing response than adding males, it is difficult to explain why the net effect in natural populations is more male production by more male-biased populations. Our short-term experimental populations might have simulated 'early season' conditions, with an absence of ephippial females, while our natural population sampling extended over the growing season and included periods when ephippial females were relatively common. Indeed, our finding of a positive effect of ASR on the likelihood of producing a male clutch lost statistical significance if we restricted our natural population analyses to samples in which no ephippial females were detected — but whether this is due to smaller sample size, or a real loss of effect, is unclear (see Table S2).

Alternatively, it is possible that the results in natural populations were driven by density, while our experimental results aimed to disentangle male and female effects and thus included strongly male-biased sex ratio treatments. As sex ratio was strongly correlated with density in natural populations, increases in relative male density coincided with increases in overall density, explaining the observed increase in male production (analogous to moving from the lower right towards the upper left edge of the surfaces in Figure 2). Additionally, our most male-biased experimental populations had ASRs of 0.75, and 22% of experimental populations had ASRs greater than 0.5 (see Table S1). While such ASR values do occur in natural populations, only 188 of our 1614 measured clutches (12%) came from natural population samples that had ASRs greater than 0.5, and only 17 clutches came from populations with ASRs greater than 0.7. Our experimental populations might therefore have better cap-

tured a hypothetical sex ratio 'switch-point' above which producing males loses value. In support of this interpretation, when analysing only the experimental populations with ASRs of 0.5 and lower, the best model no longer showed a consistent pattern of smaller male than female density effects on male production (see Table S3).

What is then left to explain is the lack of ephippia production in the extremely male-biased treatments. Female age plays a role in ephippia production: a female's early clutches are much less likely to be ephippial, although females are certainly able to produce an ephippium for their second clutch (Roulin *et al.* 2015). There may be energetic constraints on females' ability to produce an ephippium (Lynch 1983), an idea further supported by our finding that ephippia production was more likely in females that had produced relatively large asexual clutches, an indicator of good condition in *Daphnia* (Tessier and Goulden 1982; Ebert and Yampolsky 1992). Costs of ephippia production could thus contribute to the sex ratio adjustments we observed. If other individuals are reproducing sexually, a female who cannot afford to produce an ephippium can participate by asexually producing males.

Mechanistically, it remains to be determined how females detect and differently respond to the densities of females and males. In *Brachionus*, the rate at which females encounter males directly determines whether female (fertilized) or male (unfertilized) offspring are produced (Aparici *et al.* 1998). Apart from mating, *Daphnia* individuals show little direct interaction; however, they are sensitive to flow disturbances in the water around them. *Daphnia pulicaria* exhibit escape (females) or pursuit (males) behaviour at detection distances of around 4mm (males were ~1.15 mm long; Brewer 1998). Female *Daphnia* are larger than males, and the size of individuals affects the wake they produce (their 'footprint', Gries *et al*. 1999). It is not known whether this allows individual perception of the sex ratio. It is also possible that females 'count for more' in density assessments if they use more space or take up resources faster than males.

The effect of density on reproduction may also be chemically mediated: exposure to water that has previously contained dense populations increases male production (Kleiven *et al*. 1992). *Daphnia* females could conceivably produce more of the chemical cue of conspecific presence, such that a population of 10 females and 10 males would be perceived as more crowded than a population of 5 females and 15 males. Alternatively, chemicals could be sex-specific, as in copepods (Heuschele and Selander 2014), allowing for more precise estimation of the presence and abundance of different types of conspecifics in a population. Chemical sex identification appears important in the few systems in which sex ratio assessment mechanisms have been investigated (water striders, *Gerris gracilicornis*: Han *et al*. 2012; eastern red-spotted newts, *Notophtalmus viridescens*: Rohr *et al*. 2005). There is some indication that *Daphnia* males are able to chemically detect the presence of sexual females among a population of asexuals (La *et al*. 2014). Although we know of no relevant chemical study, it appears plausible that females, in turn, could use sex-specific olfactory cues to estimate the sex ratio around them. Of course, this does not preclude a role for direct physical encounters with males, who frequently attempt to cling to females.

Comparing the reproductive value of a son, daughter, or ephippial clutch is not straightforward. The returns on these reproductive investments are measured in different currencies and over different timescales (as sexually produced eggs hatch in later seasons). Facultative sexual *Daphnia* differ from obligate sexuals, which sex allocation theory has been developed for, in that allocation between male and female sexual function is not a 'zero-sum' trade-off –

increasing investment in males does not automatically reduce ephippia production, as both can increase at the expense of asexual females. Our natural data show this to occur, and additionally reveal changes in the relative frequencies of males and ephippia produced that are consistent with predictions from sex allocation theory. Our experiments support this interpretation of adaptive adjustment of offspring sex by demonstrating a causal relationship between ASR and male production, which makes up one part of the three-way allocation trade-off. Offspring sex allocation in the cyclical parthenogen *D. magna* is influenced not only by factors such as population density that drive the timing of investment in sex as a whole, but also by the current population sex ratio, which adds *Daphnia* to the list of organisms (see Introduction) that can respond to temporal variations in sex ratio by adjusting the sex of offspring they produce.

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Data accessibility

All data will be archived in the DRYAD digital repository (DOI provided on manuscript acceptance)

Authors' contributions

NG, IB, DE and HK conceived the study; NG, IB and DE designed the study; NG and IB carried out data collection, analysed the data, and drafted the manuscript; all authors revised the manuscript and gave final approval for publication.

Appendix Chapter IV

Table S1 Details of the population density and sex ratio manipulations used in three experiments.

Experiment	Manipulation	Treatment level details:				N *
		Female density	Male density	Total density	Sex ratio (% male)	
Exp1 (July 2015)	Constant density, varying sex ratio	50	0	50	0	10
		37	13	50	0.26	10
		25	25	50	0.5	10
		13	37	50	0.74	10
	Covarying density and sex ratio	50	0	50	0	8
		50	10	60	0.17	8
		50	25	75	0.33	8
		50	50	100	0.5	8
Exp2 (June 2016)	Constant density, varying sex ratio	50	0	50	0	8
		37	13	50	0.26	8
		25	25	50	0.5	8
		13	37	50	0.74	8
	Covarying density and sex ratio	25	0	25	0	8
		25	13	38	0.34	8
		25	75	100	0.75	8
Exp3 (July 2016)	Constant low density, varying sex ratio	25	0	25	0	8
		18	7	25	0.28	8
		12	12	24	0.5	8
		7	18	25	0.72	8
	Constant high density, varying sex ratio	100	0	100	0	8
		75	25	100	0.25	8
		50	50	100	0.5	8
		25	75	100	0.75	8

*** N**: number of replicate populations.

Table S2 Effects of log population density, ASR and log clutch size on the likelihood an asexual clutch contained sons; estimated by binomial GLMM with logit link. ß is the estimated change in the log odds of a male-biased clutch for a 1-unit change in the predictor. The data include only females sampled from populations where no ephippial females were detected (*N* = 578 clutches).

Fixed effects:	***ß***	**SE**	***z***	***p***
(Intercept)	-4.535	0.717	-6.33	< 0.0001
Log density	0.191	0.097	1.97	0.049
Log clutch size	0.724	0.204	3.55	< 0.001
ASR	1.380	0.930	1.48	0.138
Random effects:	**SD**			
Population ID	0.354			
Individual ID	0.00002			

Table S3 Comparison of candidate models predicting the likelihood of producing males in populations manipulating the density and sex ratio of founding adults. The data include only populations with initial sex ratios of ≤0.5.

Model	**AIC**	**Δ_i**	**Estimated parameters**
Female density, male density; experiments separate	41127	0	$-2.099 + 0.015*D_{f1} + 0.006*D_{m1} + 0.012*D_{f2} + 0.020*D_{m2} + 0.009*D_{f3} + 0.011*D_{m3}$
Female density, male density; experiments combined	41154	27	$-1.940 + 0.011*D_{f} + 0.007*D_{m}$
Total density; experiments separate	41163	36	$-1.880 + 0.009*D_{(f+m)1} + 0.009*D_{(f+m)2} + 0.008*D_{(f+m)3}$
Total density; experiments combined	41163	36	$-1.856 + 0.008*D_{(f+m)}$
Null (intercept only); experiments separate	41331	204	$-1.183 + D_{(f+m)1} + D_{(f+m)2} + D_{(f+m)3}$
Null (intercept only); experiments combined	41354	227	$-1.183 + D_{(f+m)}$

CHAPTER V

Abandoning the Ship using Sex, Dispersal, or Dormancy: Multiple Escape Routes from Challenging Conditions.

Sleeping? Leaving? Sex?

The ship might sink tomorrow

Complex decision

Nina Gerber & Hanna Kokko. *In preparation* for *Philosophical Transactions of the Royal Society B*.

Abstract

Natural populations often experience environments that vary across space and over time, leading to spatiotemporal variation of the fitness of a genotype. If local conditions are poor, organisms can disperse in space (physical movement) or time (dormancy, diapause). Facultatively sexual organisms can switch between asexual and sexual reproduction, and thus have a third option available to deal with maladaptedness: They can engage in sexual reproduction in unfavourable conditions (an 'abandon-ship' response). Sexual reproduction in facultatively sexual organisms is often coupled with dispersal and/or dormancy, while bet-hedging theory at first sight predicts a negative relationship, as different escape mechanisms could substitute for each other. Here we briefly review the observed links between sex, dormancy and dispersal, and model the expected covariation patterns of dispersal, dormancy and the reproductive mode in a context of local adaptation to spatiotemporally fluctuating environments. The sign of correlations between sex, dormancy and dispersal evolve to be different in within-species vs. across-species patterns. Various risk-spreading strategies are not completely interchangeable, as each of them has dynamic consequences that can feed back into the profitability of another. Our results shed light on the discrepancy between theoretical predictions on covarying risk-spreading traits and help explain why sex often associates with other means of escaping unfavourable situations.

Introduction

Natural populations often experience environmental challenges that vary across space and over time, which leads to spatiotemporal variation in fitness components of a given genotype. Spatiotemporal variation is one of the key factors behind dispersal (Ronce 2007; Starrfelt and Kokko 2012*a*), but moving elsewhere is not the only way to escape a situation that may pose problems of survival or reproduction. Just like dispersal in space can spread the risks that a lineage encounters across space, 'dispersal in time' — i.e. dormancy or diapause — can perform the same task across a temporal dimension (Levin et al. 1984*a*; Vitalis et al. 2013*a*; García-Roger et al. 2014) in those organisms capable of surviving unfavourable periods of time using this method.

Some organisms also have a third option available to them: a choice of whether reproduction occurs sexually or asexually. Facultatively sexual organisms (often also called cyclical parthenogens) often engage in sex when they encounter stressful conditions (see (Ram and Hadany 2016) for a recent review). To understand why sex can work somewhat analogously to dispersal or dormancy, it is useful to view each of these strategies as mechanisms with which to escape current conditions that are challenging to cope with. An organism that avoids dispersal, avoids dormancy, and avoids sex (i.e. reproduces asexually) is committed to creating exact copies of itself to utilize the current resources. While this can conceivably pay off when conditions are both good and unchanging, any other situation might call for risk spreading strategies. An allele that promotes dispersal will find itself in novel spatial locations, an allele that promotes dormancy will be expressed in a new environment some time into the future, and an allele that promotes sex will find itself placed into a new, and hopefully better performing, genetic background. Thus sex adds a 'travel in identity' option to the commonly contrasted set of travel in either space or time. This perspective on sex has been called the 'abandon-ship' principle (Hadany and Otto 2007; 2009; Schoustra et al. 2010; Griffiths and Bonser 2013), and predicts that organisms in poor condition should be particularly prone to choosing a sexual over an asexual life-cycle. More generally, each of the 'travel' strategies can conceivably represent bet-hedging (Vitalis et al. 2013*a*; Li et al. 2017), defined as strategies that lead to beneficial reductions in fitness variance (Slatkin 1974; Starrfelt and Kokko 2012*b*) while reducing mean fitness (reflected, respectively, in discussions of costs of sex (Lehtonen et al. 2012), costs of dispersal (Bonte et al. 2011), and mortality during dormancy (Cohen 1966; Hopper 1999).

Spatiotemporal environmental variations are not the only factors that select for dispersal, dormancy or sex (for general reviews see e.g Linkies et al. (2010), Clobert et al. (2012), Hartfield and Keightley (2012), Lively and Morran (2014)). Yet, it presents a particularly interesting set of challenges because it can, in principle, select for 'travel' of one or the other kind, perhaps simultaneously. This raises the question which set of mechanisms an organism is expected to employ. A general expectation from bet-hedging theory (Starrfelt and Kokko 2012*b*) is that efficient use of one method should diminish selection to employ any other (heuristically put, it is not worth paying twice to solve the same problem once).

Dispersal and dormancy as alternatives that might substitute for each other has attracted theoretical and empirical attention (Snyder 2006; Vitalis et al. 2013). These strategies share similarities in entailing morphological or other costs (e.g. predation risk, (Vitalis et al. 2013), or the risk of ending up in an unknown environment, (Snyder 2006)). Their benefits are

similarly largely analogous (Venable and Brown 1988; Venable et al. 1993), including reducing crowding (Levin et al. 1984), local competition among relatives (Hamilton 1963, Taylor 1988, but see Eberhart and Tielbörger (2012)) and reduced inbreeding (Gandon 1999; Roze and Rousset 2005). If these strategies substitute for each other (Cohen and Levin 1987; Venable and Brown 1988, Philippi and Seger 1989, Tsuji and Yamamura 1992, Olivieri 2001, Vitalis et al. 2013, Buoro and Carlson 2014), one expects a negative correlation between them, species or populations with stronger dormancy investing less in dispersal (evidence from plants supports this interpretation, though not without exceptions (Buoro and Carlson 2014). As pointed out by Snyder (2006), exceptions can occur because dispersal and dormancy are not dynamically equivalent, but possess intertwined fitness effects: assuming that the natal habitat is good enough to have produced a newborn (and thus likely better than a random site), together with some positive temporal autocorrelation, dispersal means moving to a place that does not enjoy the statistical association between 'currently good habitat' and 'natal site'. Dormancy makes this cost smaller, as the association at home becomes weaker due to the temporal spread of the organism's active life at the natal site. This can create positive covariation between dispersal and dormancy (Snyder 2006).

Less theoretical attention has been paid to the patterns between sex on the one hand and dormancy and/or dispersal on the other. Empirically, however, patterns of covariation are amply documented, which we briefly review below. Our subsequent goal is to clarify expected covariation patterns between facultative sex, dormancy and dispersal, when investment in these strategies can depend on the current condition. We highlight that the within-species patterns may differ from across-species patterns.

What patterns are there?

Observed links between dormancy and dispersal have been reviewed elsewhere (Buoro and Carlson 2014), thus we focus on the question of sex (in facultative sexual organisms) and its co-occurrence with dispersal and/or dormancy.

Sexual reproduction (in facultative sexuals) has long been reported to associate with dispersal, the formation of resistant structures, or both (Bonner 1958; Abrahamson 1980; Castel et al. 2014). When sexually produced offspring possess traits that deviate greatly from asexually produced ones, this is often in the direction of more hardiness: for example, in most aphid species, sexually produced eggs are the only cold-resistant stages (Simon et al. 2002). In the cladocerans *Daphnia* (Tessier and Caceres 2004; Ebert 2005) and *Bythotrephes* (Wittmann et al. 2011), sexually produced eggs undergo diapause, encapsulated in a cold- and drought-protective envelope. The same applies to cyclically parthenogenetic rotifers (Carmona et al. 2009). Oligochaete worms can reproduce asexually via fission, whereas diapausing cocoons are produced sexually and are induced in unfavourable conditions (Schierwater and Hauenschild 1990). In many plants, growth can lead to vegetative propagules, whereas seeds tend to be produced sexually. Also, in those fungi that can produce both sexual and asexual spores, the latter appear more environmentally resistant while asexual spores are destined to germinate quickly (Aanen and Hoekstra 2007).

However, there are also exceptions. Within already mentioned taxa, some *Daphnia* (Innes et al. 2000) are capable of producing resting stages asexually, and apomictic plants can produce seeds asexually. Likewise, although the general pattern in fungi can be summarized as survival structures associating with sex (e.g. meiotically produced ascospores, (Nieuwenhuis

and James 2016)), many species also have the ability to produce conicia, asexual survival structures that can also disperse (Worrall 2009). Also, there are taxa (e.g. tardigrades (Welnicz et al. 2011), bdelloid rotifers (Caprioli and Ricci 2001)) where adult stages can enter an anhybrobiotic stage, in which they are able to endure extreme conditions; and finally, certain bryozoans produce statoblasts, masses of cells contained in a chitin shell, that can withstand desiccation and freezing (and can either disperse or stick to the parent colony (Ruppert et al. 2004)).

The association of dispersal (movement in space) with sexual reproduction (Bell 1982) appears similar, i.e. a relatively clear pattern comes with intriguing exceptions. First, although not usually phrased as an explicit sex/asex contrast, vegetative growth in modular organisms (e.g. many plants) can lead to offspring that can be considered independent in demographic studies (Martínková and Klimešová 2017) — runners in strawberries being a familiar example. Such offspring typically do not end up growing far from their parent, while sexually produced seed may be equipped with traits that ensure dispersal. This should not be taken as so self-evident that it warrants no further thought, since no biological law prevents vegetative growth from continuing from the point onwards where fertilization occurred (or, conversely, dispersive structures being formed asexually). For example, in many fungi, a fertilized mycelium can keep dividing, taking its fertilized state — called a dikaryon — with it.

Most basidiomycetes (where mushrooms and puffballs belong to) disperse primarily as haploid basidiospores. In mushroom-forming fungi, sex and reproduction (via spores) are temporally and physically separate. Fertilization happens underground, where two monokaryons — haploid mycelia — meet; fertilization leads to a dikaryon, with the dikaryotic state then spreading through existing mycelia [40]. Spores are produced by the dikaryotic mushroom. The continued post-fertilization growth of mycelia forms a clear contrast to plants. Still, one can state there to be some association with sex and long-distance dispersal, in the sense that dispersal through meiotically produced spores follows the underground sexual bout, though with a clear delay (Zeyl 2009, Billiard et al. 2012). In some pathogenic fungi, sex is also required for the production of infectious spores to colonize a new host (Heitman 2010, Billiard et al. 2012). In contrast, plant fertilization, with pollen travelling to the stigma, features a harder to break connection between dispersal and sex: this type of gene flow appears unavoidable in any outcrossing plant. To the extent that seed production requires sex (apomictic plants present an exception), sexual reproduction and dispersal become intrinsically coupled.

The more efficient dispersal of sexually produced structures (or, generally, offspring) than vegetatively grown ones also occurs in lichen-forming fungi: morphological features that can lead to asexual dispersal are, among others, peg-like isidia, or leaf-like squamules or phyllidia; this is not an exhaustive list of all shapes that can easily break away and can be dispersed (Nash 1996, Smith et al. 2009). This type of dispersal contrasts with sexually produced ascospores, which only contain the fungal part of the algal-fungal symbiosis. The ascospores are actively discharged into the surrounding air ('fungal cannons' *sensu* Trail (2007)) to enhance wind dispersal. Again, sexually produced offspring appear designed to disperse further than asexual propagules (Gregory 1945, 1961). Lichen reproduction is thus argued to provide genetic and spatial escapes from kin competition (Hestmark 1992).

In other fungi, the size and shape (and therefore dispersal ability) of the propagules is often dependent on the reproductive mode. Some fungi produce two types of spores: for instance,

Myophelaijen produces ascospores sexually and conidia asexually. While ascospores are dispersed by wind from several hundred metres to several kilometres, conidia travel by rain-splash up to a few metres only (Halkett et al. 2010; Rieux et al. 2014). Also other sexual structures, such as basidiocarps, enhance spore dispersal through wind, water or animals (Billiard et al. 2012).

In sessile and modular animals, active dispersal is often associated with a switch from vegetative to sexual reproduction (Bell 1982), but there is also passive dispersal which does not require sex: in most hydroids, bryozoans, corals and polychaeta, asexual reproduction is achieved by units that separate from parents by budding, fission or by accidental fragmentation. These units may disperse passively. Active dispersal, however, is achieved by sexually produced larvae (Gravier-Bonnet 1992; Ryland 2006). Similar patterns are found in sponges (Porifera), where asexual reproduction occurs by budding, gemmulation or fragmentation (Fell 1993, Maldonado and Riesgo 2008), leading to passive dispersal via currents and storms (Fell 1989) . Porifera sex results in the production of free-swimming, actively dispersing larvae; gametogenesis is triggered by environmental factors, e.g. temperature (Fromont and Bergquist 1993, Maldonado and Riesgo 2008).

In many Cnidaria classes (Siphonophora (Fautin 2002), Trachylinae and Scyphozoa (Ceh et al. 2015), Anthozoa (Harrison and Wallace 2006)), sexually produced offspring typically engage in active dispersal, (Bell 1982, Hestmark 1992). In Ctenophora (e.g. entoprocts; commonly known as comb jellies), asexual reproduction takes place through budding; the resulting 'offspring' can swim slowly — this group is the largest animal that swims using cilia — but with unknown dispersal distance (Sugiyama et al. 2010). Sexually produced larvae might disperse over far distances, even though exact distances are unknown (Sugiyama et al. 2010).

Echinoderms (e.g. sea stars and sea urchins) reproduce asexually via fission, where an arm or another part of the body can regrow after breaking off (Fisher 1925). Some species engage in active asexual reproduction. A fracture develops on the lower surface of the arm and the arm pulls itself free from the body, which holds onto the substrate (Monks 1904, McAlary 1993). Larvae, which can disperse over much longer distances, are usually sexually produced and develop after broadcast spawning, but larvae can, very rarely, arise through parthenogenetic reproduction (Yamaguchi and Lucas 1984). In both main classes of Hemichordata, there is a similar pattern. Enteropneusta (acorn worms) engage in vegetative reproduction after an adult worm breaks into two, and Pteroanchia can bud asexually (Hadfield 1975). Both classes can also reproduce sexually and produce actively dispersing larvae.

Finally, it is worth commenting on an analogy between selfing/outcrossing and asex/sex. Although self-fertilization is not equivalent to full asexuality, there are similarities between our question and the much better addressed botanical question of whether self-fertilization should be associated with less, or more, dispersal than outcrossing. Work to date (Cheptou and Massol 2009, Massol and Cheptou 2011, Iritani and Cheptou 2017) has produced intriguingly conflicting results: models that do allow the same individual to produce two different dispersal morphs find that dispersal ability should covary positively with outcrossing (Cheptou and Massol 2009, Massol and Cheptou 2011); the opposite pattern becomes more likely (though with the answer depending on whether one assumes evolutionary constraints to play a role or not) if a plant can equip its selfed seeds with different dispersal abilities than the outcrossed ones (Iritani and Cheptou 2017).

In the vast majority of the above documented cases, the likely dispersal distances are not known to any degree of precision. The allocation decisions, including the cues that an organism uses to begin investing in sexual reproduction, are typically also not known to any great detail — with the exception of cyclical parthenogens. Here condition-dependent investment is often documented (Hadany and Otto 2009, for a review see Ram and Hadany (2016)): sex is triggered by nutrient limitation in a large number of microbial species (e.g. *Chlamydomonas* (Umen and Heitman 2013)) as well as in multicellular Metazoa. Increasing crowding, which typically implies reduced food availability, as well as extreme temperatures induce male production and sex in *Daphnia* (Kleiven et al. 1992). Indeed, it has been pointed out that sex becomes easy to explain if sex is a prerequisite to the production of a dormant form, and time periods that can only be survived using dormancy occur regularly (Stelzer and Lehtonen 2016). The deeper question, however, is why sex should be associated with the production of such forms in the first place.

Here we model coevolutionary dynamics where populations can locally adapt to their environment, investing independently in sexual reproduction, dispersal and dormancy in spatially and temporally varying environments.

Model

Overview

We created individual-based simulations of a population of facultatively sexual diploid hermaphrodites inhabiting a toroid shaped world. Each individual has 13 diploid loci in total. 12 of them result from having two alleles each for four parameters that are required to describe a logistic reaction norm for three independently evolving traits: sex, dispersal and dormancy. The remaining 2 loci describe an individual's phenotype in terms of its adaptedness to the current environment (see 'Environment' below for details).

We assume that individuals are able to measure their adaptedness to the current environment, as the degree of mismatch (denoted θ) is impacting the individual's condition. Condition is measured implicitly, with larger deviations between phenotype and the ideal phenotype (the one currently favoured by the environment) implying poorer condition. Each reaction norm is described by four evolving parameters, a, b, c and d (with both a and b bounded between 0 and 1), giving flexibility allowing an individual to respond to its current levels of maladaptedness, θ, by either increasing or decreasing the probability of each response (sex, dispersal, or dormancy). Options include keeping the response always highly probable, or always highly unlikely (Figure 1); this is achieved by using the logistic functions for each probability

$$p_{sex} = a_{sex} + \frac{b_{sex} - a_{sex}}{1 + e^{-10c_{sex}(\theta - d_{sex})}} \quad (1a)$$

$$p_{disp} = a_{disp} + \frac{b_{disp} - a_{disp}}{1 + e^{-10c_{disp}(\theta - d_{disp})}} \quad (1b)$$

$$p_{dorm} = a_{dorm} + \frac{b_{dorm} - a_{dorm}}{1 + e^{-10c_{dorm}(\theta - d_{dorm})}} \quad (1c)$$

Here θ describes the match between an individual and the state of the environment it resides in, defined such that θ is the distance to the environmental state (the locally optimal phenotype). Thus increasing θ implies increasing maladaptedness. The parameters are additively

determined by the sum of allelic values at each locus (see '*Mode of reproduction, inheritance & mutations*' for details). Note that as we do not constrain b to exceed a, nor require c or d to remain positive, thus the model allows individuals of low condition to evolve either higher or lower probabilities to perform an 'escape' compared with an individual in high condition.

We model soft selection, such that each patch creates the same number of offspring in each generation (denoted φ), with better-adapted individuals (that are not currently undergoing dormancy) disproportionately represented as parents.

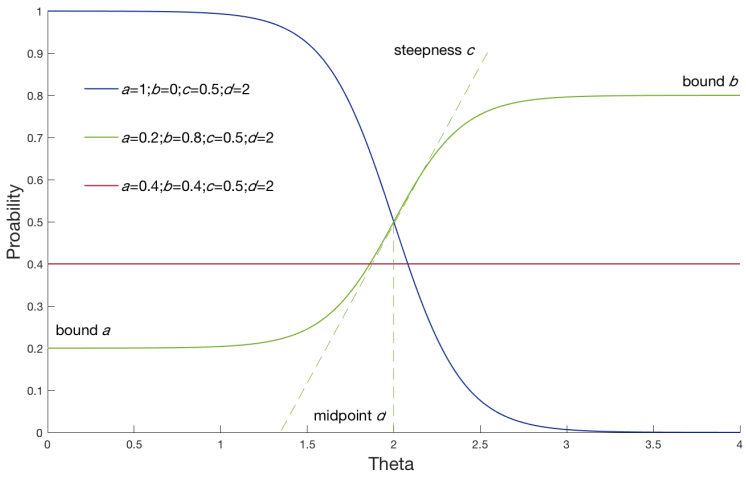


Figure 1 Example reaction norms for different combinations of *a,b,c,d.*

The environment

The population inhabits a 2-dimensional world of $S \times S$ discrete habitat patches, with wrapped edges (i.e. a toroid world in which moving to a smaller patch number from patch 1 along either axis leads to patch S). Each patch, characterized by coordinates i and j, has an environmental state $E_{ij}{}^t$, bounded between 0 and 1, that varies over time t. We initiated the world at time $t = 0$ by setting $E_{ij}{}^0$ as either 0 or 1 with 50% probability (no spatial autocorrelation). Spatial and temporal autocorrelation were thereafter introduced by updating the environmental state using temporal and spatial autocorrelation mofidiers p_t and p_s, respectively, such that

$$E_{ij}^{t+1} = p_t(p_s\overline{E_{ij}} + (1 - p_s)E_{ij}^t) + (1 - p_t)\varepsilon \qquad (2)$$

Here $\overline{E_{ij}}$ is the mean of the environmental states of the four neighboring squares; ε is a uniformly distributed random number between 0 and 1 (thus if p_s is low, patches tend to deviate greatly from their neighbours; if p_t is low, the newly randomized number ε impacts the environmental state more than the recent history). Each patch has the same number of neighbours (the neighbours of 11 are S1, 1S, 12 and 21).

The resulting dynamics of the $S \times S$ grid was normalized so that the mean E_{ij}^t averaged across patches was, at all times, 0.5 with standard deviation σ, to avoid creating worlds that are inherently more difficult to adapt to in any other sense than varying the temporal and spatial rate of changes.

Optionally, each time step has patches becoming temporarily uninhabitable with probability *f*, independently applied to each patch. An uninhabitable patch kills all active individuals, while dormant individuals follow normal mortality rules (for dormancy), with no hatching attempts, during a period of uninhabitability. Once patches recover from being uninhabitable, their environmental state is calculated as if they never had been unsuitable. A restored patch can be recolonized through hatching of dormant eggs and/or dispersal.

Initializing the population

We initialized the population by placing a total of φS^2 individuals (i.e. the expected global output if all patches are habitable; this also equals the maximal number of active individuals at any given time point) at random locations, i.e. with uniformly distributed *i* and *j* coordinates between 1 and *S*. Alleles were initiated with uniform distributed random numbers between 0 and 0.5 for *a* and *b*. The phenotype, *c* and *d* were initiated as random numbers drawn from the standard uniform distribution on the interval (0,1) and divided by 2, such that the sum of two alleles falls between 0 and 1.

Sequence of life history events within a generation

Every generation is, without loss of generality, assumed to take 1 year (though dormant individuals can live longer) and has the following temporal order. Every year begins with a fraction τ of dormant individuals dying, regardless of their patch being uninhabitable or not. All non-dormant inhabitants of currently uninhabitable patches die. Thereafter, we examine the following events.

Activation of dormant individuals; measurement of local (mal)adaptedness

On each patch, local dormant individuals can re-awaken. The number of such individuals may be limited either by the availability of dormant individuals and the rate at which they return to the active state, or by density-dependent factors limiting newcomers' ability to recruit locally. We therefore take the number of awakening individuals as the smaller number of wn_{ij} and $\varphi - N_{ij}$, where *w* scales the duration of dormancy (high *w* leads to short dormancies), n_{ij} is the number of local dormant individuals, and N_{ij} is the current number of non-dormant individuals. The choice of the awakening individuals among the local dormant ones is random.

Each non-dormant individual has its maladaptedness score θ determined as $\theta = \left|E_{ij}^{t} - T\right|$ where *T* is the sum of the individual's two alleles that determine the phenotype.

Mode of reproduction, inheritance & mutations

Active (non-dormant) individuals opt for being in the category of sexuals with probability given in eqn. (1a); those who do not opt for sex are categorized as asexuals. However, if there is only one individual on the patch, it will reproduce asexually regardless of its traits.

The patch produces a total of φ offspring, which can be produced sexually or asexually. Each offspring is first assigned a mother: all individuals 'compete' to be chosen as the mother, with propensities $e^{-\theta^2}$ for sexual individuals and $\alpha e^{-\theta^2}$ for asexual ones to be chosen (if α = 2 we are assuming a twofold cost of sex). For offspring with a sexual mother, a father is chosen, with a similar procedure but now both sexual and asexual individuals have propensity

$e^{-\theta^2}$ to be chosen (we thus assume that asexual hermaphrodites can participate in siring sexual young via their male function), except for the mother of the offspring, whose propensity is now 0 (i.e. we exclude selfing). Apart from no selfing, we do not change individuals' propensities based on the fecundity they have already reached, thus multiple mating is allowed both in the male and in the female role.

Asexually produced offspring are created as copies of their mother. Sexually produced offspring obey Mendelian inheritance for all traits with no linkage assumed between any of the loci. Every allele then has an independent probability μ of mutating; mutations change the allelic value to either a lower or a higher value (50% either direction) by adding normally distributed random numbers to alleles being mutated. Mutated alleles with values > 0.5 are set to 0.5, while negative values are set to 0, to keep the sum of two alleles bound between 0 and 1.

Dispersal & dormancy

Offspring disperse and/or go dormant according to eqns (1b)-(1c). There are several options, depending on whether we assume that offspring base their decision on their own maladaptedness or that of their mother (a maternal cue), whether the reaction norm parameters are determined by offspring or parental alleles, and how an offspring is assumed to behave if eqns (1b-c) tell it to both disperse and to go dormant. We use two alternative sets of rules. In the first set, we assume that mothers make these decisions for their offspring (thus we use the mother's θ as well as the mother's reaction norm parameters for eqns 1b-c), and that an offspring who is 'told' (by the mother) to both disperse and go dormant is able to do both (i.e. it disperses and then becomes dormant in its new patch — a biological possibility is e.g. a winged seed of a plant). In the second set, we assume that offspring make the decision about dispersal and dormancy based on their own reaction norm parameters and θ. Note that the dormancy decision is made based on the θ in the new environment, in case the offspring dispersed. If offspring disperse to an unsuitable patch, its θ becomes infinite.

Dispersal is assumed risky: a fraction $1-\eta$ of dispersers survive. Survivors are assumed to land in a random direction (any real number angle between 0 and 2π) from their original [[*i*,*j*]] coordinates, with a distance drawn from an exponential distribution with mean D. An individual's new patch is determined by rounding the coordinates to the nearest integer, interpreted within the toroid geometry; thus e.g. post-dispersal coordinates of [[7.81, 22.17]] are interpreted as patch [[8, 2]] if the world consists of 400 patches (S = 20).

Equation (1c) is then applied to all offspring to determine if they go dormant (again depending either on their mother's reaction norm in the environment in which the offspring was produced or on their own reaction norm in the new environment, depending on the rule). We set a limit to the size of the 'seed bank', i.e. the bank of dormant individuals: this consists of 10 times the maximal number of active individuals. If the maximum seed bank size is reached, entering dormancy is still possible, as random dormant competitors get replaced. The rules of ending dormancy are part of the next generation, already explained above.

The offspring generation now replaces the parental one, which completes the annual cycle; the environment then changes state, and a new year begins.

Simulations

Simulations were performed for t_{max} years, long enough to make dispersal, dormancy and sex probabilities stabilize (example shown in Figure 2). We examined results (i) assuming only one escape mechanism evolves at a time (named 'constraint' simulations as two escape mechanisms are set to a fixed level), or (ii) assuming all of them coevolve ('joint evolution' simulations). In the former case, we fix two of the three reaction norms such that there is no condition-dependence in them; practically, this is achieved by setting $a = b$ and the relevant mutation probabilities μ to 0. We then follow the evolution of the remaining trait, while varying the non-evolving traits systematically in the [[0, 1]] range. In the joint evolution simulations, every trait is allowed to evolve, while we systematically vary the cost of sex ($1 \leq \alpha \leq 2.5$), cost of dispersal ($0 \leq \eta \leq 0.9$), and cost of dormancy ($0 \leq \tau \leq 0.9$) yielding a total of 120 'species' varying in all of these 3 parameters; we then proceeded to examine within-species and across-species patterns within this hypothetical dataset.

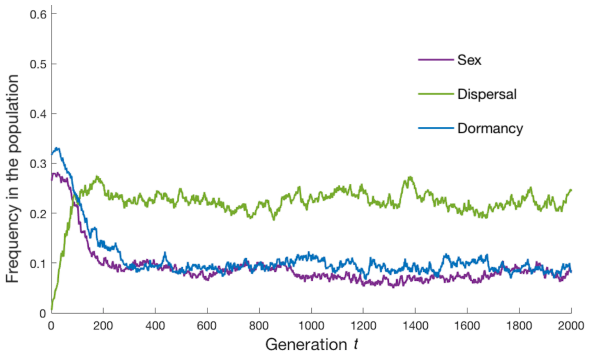


Figure 2 Example of the evolution of the mean frequency of sex, dispersal and dormancy within a population in the joint trait evolution simulations. α = 2, η=0.1, τ =0.1, w= 0.8, D=1, ps=0.9, pt=0.9, $t$max=2000, f=0.

For both simulations we report the outcomes, distinguishing between high (we use $p_s = p_t = 0.9$) or no ($p_s = p_t = 0$) spatial and temporal autocorrelations, as well as for the rule sets 'offspring decides' and 'mother decides', combined with the optional scenario where patches can become unsuitable ('ephemeral environment' scenario) or not ('continuous variation' scenario). These yield 2 × 2 × 2 × 2 options to examine (Figure S1). We report the probabilities of engaging in the three escape mechanisms (sex, dispersal, dormancy), as predicted by eqns (1a)-(1c), separately for well adapted individuals (those with maladaptedness scores that are smaller than the global median θ_{50}) as well as for poorly adapted individuals (those with maladaptedness scores that exceed than the global median). All results present averages over 10 independent runs.

Results

'Constraint' simulations

In the constraint scenario, one trait is allowed to evolve while the other two are varied systematically (and kept constant within one run of a simulation). Unless specifically mentioned, there was no qualitative difference between the 'Mother decides' (Figure 3) and 'offspring decides' scenario (Figure S2).

Sex and dormancy decrease with dispersal rate; dormancy leads to higher dispersal

If sex is free to evolve in response to the rates of dispersal and dormancy, the frequency of sex decreases with increasing dispersal rate, with little systematic change with the rate of dormancy (Figure 3a). There is no qualitative difference between environments with high vs. no spatio-temporal autocorrelation (Figure 3a). In all cases, the frequency of sex remains relatively low (less than 10% in the examples of Figure 3a). Very low levels of dispersal in ephemeral environments lead to extinctions (Figure S9a).

The frequency of dispersal increases at high rates of dormancy, with very high dispersal rates reached when dormancy rate 1 is enforced; the rate of sex has little effect on dispersal (Figure 3b). This result is maintained across different spatiotemporal variation patterns, though the frequency of dispersal increases in environments with no spatio-temporal auto-correlation (i.e. fast changing environments). Ephemeral environments, likewise, increase the frequency of dispersal compared with continuously varying environments (Figure S9b).

When the frequency of dormancy is free to evolve, we find a contrasting outcome: while dispersal evolved to be high when dormancy was kept high (discussed above), the converse is not true: dormancy evolves to be high when dispersal is *low*. Again, the rate of sex does not systematically change the pattern (Figure 3c), and there is no qualitative difference between environments differing in spatio-temporal autocorrelation. The 'continuous variation' scenario (Figure 3c) and the 'ephemeral environment' scenario (Figure S9c) are similar in all these respects.

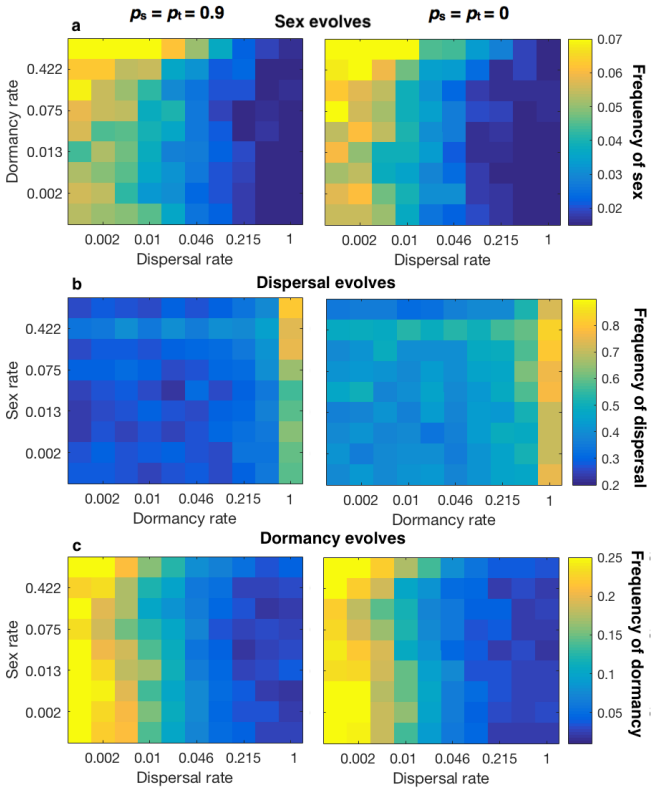


Figure 3 'constraint' simulations: Mean frequencies, measured at the end of 10 independent runs, of (a) sex, (b) dispersal and (c) dormancy, depending on the rate of the two other non-evolving traits (see Figure S2 for exact values); note the different scales on the colorbars for each trait. Left panels, high spatial autocorrelation; right panels, no spatiotemporal autocorrelation. All figures are based on the 'mother-decides scenario' (see Figure S2 for offspring decides scenario) with continuous variation; $\tau = \eta = 0.1$, $\alpha = 2$, $w = 0.8$, $D = 1$.

'Joint evolution' scenario

Across-species predictions: High dispersal decreases frequency of sex and dormancy; positive covariation between sex and dormancy

Here all traits are allowed to evolve, and we vary the cost of dispersal, dormancy and sex (which presumably can vary across species in nature). The corresponding trait evolves as expected, e.g., species with high cost of dispersal (indicated by bluer symbols in Figure 4) evolve to disperse less often. Simultaneously, the cost of dispersal affects the frequency of sex and dormancy. High cost of dispersal, by decreasing dispersal itself, increases the frequency of sex and dormancy in the population (Figure 4; an expected pattern given the influence of dispersal that was forced to be high or low in Figure 3). Based on Figure 3, we expect dormancy to have less strong effects, unless it evolves to high rates, where it has a positive effect on sex as well as on dispersal. Indeed, very low dormancy costs can slightly decrease the frequency of sex but increase the frequency of dispersal (FigureS4). This increased frequency of dispersal at low cow cost of dormancy is even more pronounced in the 'offspring decides' and the 'ephemeral environment' scenario (Figure S8).

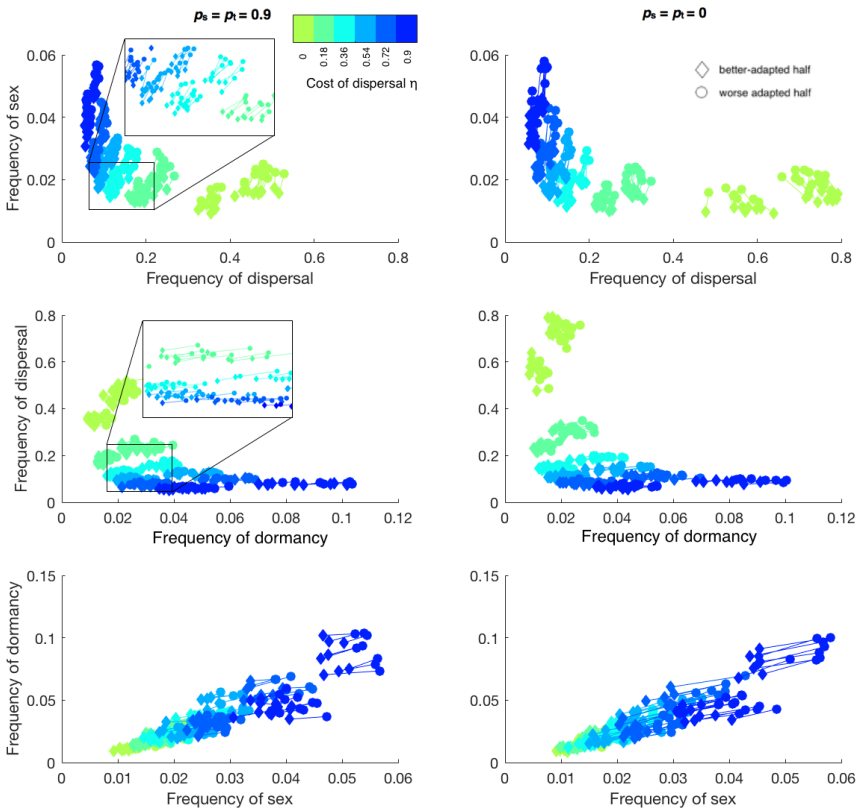


Figure 4 'joint evolution ' simulations: Within- and across-population correlations of (a) dispersal and sex, (b) dispersal and dormancy and (c) sex and dormancy. Each connected pair of a diamond with a filled circle represents a population. Different populations differ in η, τ and α. The colours indicate the cost of dispersal, η (for visualizations that allow to depict dependencies on other costs, τ or α, see Figure S3-4). Each symbol pair represents the better-adapted individuals (diamonds) and the poorly adapted individuals (circles). Columns indicate the different scenarios with high spatiotemporal autocorrelation (left) and no spatiotemporal autocorrelation (right). All panels show the results for the 'mother decides scenario' in continuously varying environments (see Figure S5-S7 for other scenarios). For all panels w =0.8, D =1 and f=0.

Based on Figure 3, we can also predict that the frequency of sex has at best a mild effect on the other traits; indeed, in the joint evolution scenario, the cost of sex does not influence the frequency of dispersal or dormancy (Figure S3). Interestingly, the evolved frequency of sex itself showed much clearer responses to the cost of dispersal (Figure 4) and the cost of dormancy (Figure S4) than to the cost of sex itself (Figure S3).

To phrase the findings in terms of positive and negative correlations: when comparing the frequency of sex with the frequency of dispersal across species, we find a negative relationship between the two traits, such that highly sexual species invest less in dispersal (Figure 4a). Similarly, dormancy and dispersal covary negatively (Figure 4b). However, the across-species patterns of the frequency of sex and the frequency of dormancy is positive (Figure 4c). This appears to be driven by the strong effect of the cost of dispersal on all traits, i.e., when costs of dispersal are prohibitive, high frequencies of sex as well as dormancy evolve to compensate.

The above statements are robust with respect to continuously varying vs. ephemeral environments, (Figure 4, S5, S6, S7), or scenarios where 'mother decides' (Figure 4, S5) or 'offspring decides' (Figure S6-S7). Quantitatively, however, the 'ephemeral environment' scenario leads to generally higher investment in escape traits, the difference being pronounced in high autocorrelation environments (Figure S5 & S7).

Within-species predictions: Condition dependent investment leads to positive correlation of sex, dispersal and dormancy

Across all scenarios considered, poorly-adapted individuals engage more in sex, dispersal and dormancy than well-adapted individuals, leading to a within-species expectation that these traits are linked (Figures 4, S4-S7: circles are almost invariably located more to the right, and higher, than diamonds). In special cases (at very low cost of dispersal and no spatiotemporal autocorrelation), the difference in dispersal was found to disappear or even reverse while the within-species positive correlations between sex and dormancy were maintained (e.g. Figure 4).

Discussion

Our study views dispersal, dormancy and sex as three alternative (but potentially co-occurring) escape mechanisms, in the sense of the options available to an allele residing in a maladapted body: escaping spatially, temporally or genetically can all potentially restore high fitness, but each also represents a jump into the unknown. Our main finding is that the sign of the expected correlation between these three escape mechanisms can switch between within-species patterns and across-species patterns.

We discuss within-species patterns first. A previous study has, on the conceptual and empirical front, suggested that mechanistic trade-offs between the ability to disperse and the ability to go dormant create negative covariation patterns (Buoro and Carlson 2014); our model is simpler in the sense of incorporating no such trade-offs, in which case the prediction shifts to a positive correlation between all three traits. In the absence of a tradeoff that would effectively force each individual to specialize, our model predicts that dormancy, dispersal and sex (or two of them) can be employed simultaneously. All these responses are, in our model, based on condition-dependence. A scenario of polymorphic specialization (where one individual goes for dormancy, another for sex and a third disperses) would require that a poorly

adapted individual solves its problems via one escape mechanism so perfectly that a well-adapted individual is left to do more escaping via another means. This appears unlikely, both intuitively and according to our results. While classical bet-hedging theory can produce diversified specialists, it does not consider plasticity based on local adaptedness, or condition. When responses are based on reaction norms (as in our model), our results suggest that specific individuals of one species will express an 'escape syndrome' that employs multiple routes at once. Cases where within-species variation is negative (for plant and insect examples see (Buoro and Carlson 2014)), then, are conceivably indicative of trade-offs that make it difficult to possess all traits at once required for successful multi-route escaping.

Our probabilistic way of modelling escape probabilities via three independent functions has some consequences for the interpretation. When probabilities are applied independently, populations are bound to have individuals expressing any possible combination (some show asexuality, dormancy, and no dispersal; others differ in just one trait from this, etc). Given the positive correlation emerging between them, however, we can conjecture that a pleiotropic mutation that causes two (or even three) of the phenotypic changes simultaneously, in the same reaction norm, has a chance to spread. We therefore expect adaptations that cause a switch from asex to sex while also causing survival or dispersal structures to form. Future work could usefully consider whether such pleiotropy would win over alleles causing just one reaction norm to change at a time. This, potentially together with an explicit examination of trade-offs, could shed further light on the obligateness of connections between sex and dispersal in time or space.

Turning to across-species patterns, here we showed the potential for more diverse patterns than the simplest interpretation from bet-hedging theory would predict (Starrfelt and Kokko 2012*b*). This does not make a bet-hedging view useless: it creates the *a priori* prediction that traits *might* substitute for each other, and indeed our results confirm this can happen. Some of our predictions are, to our knowledge, novel: not only dormancy (discussed elsewhere) but also sex — when facultative — should respond to dispersal. If dispersing is, for one or another reason, difficult in a given species, its rate of sex should increase. A detailed look at local adaptation can potentially explain this, as well as the challenging task to explain positive across-species covariation patterns, given an a priori expectation of a traits maybe substituting for each other. Maximal (obligate) sex, or obligate dormancy, each lead to stronger local adaptation (shown in values of theta clustering more strongly around zero, Figure S10), than high dispersal. Thus, dispersal, with its gene flow that 'swamps' local adaptation (Lenormand 2002) , has stronger potential to create subpopulations with many locally maladapted individuals. Additional heuristic insight is provided by the concept of 'genetic time travel' (Engelstadter and Moradigaravand 2013) in a model of bacterial transformation, where recurrent environmental change can make it beneficial for individuals to uptake DNA that arose in the past in a different lineage than their own. Costly sex in our model, likewise, appears to pay off more when some currently active individuals were born some time ago.

Thus, our results are in line with earlier work (Snyder 2006; Vitalis et al. 2013*a*) showing that various risk spreading strategies are not completely interchangeable: each of them has dynamic consequences that can feed back into the profitability of another. In the 'constraint' scenario, forcing high rates of dispersal makes the frequency of dormancy decrease (negative relationship), but the converse is not true (high dormancy leads to high dispersal; a positive relationship). Dispersing seeds risk of landing in currently unfavorable patches; dor-

mancy can reduce this cost, by spreading the germination time of theses seeds (Snyder 2006). However, because the response is only clear at very high dormancy rates, we do not find an increase of dispersal with the rate of dormancy (i.e. increased dispersal at low cost of dormancy in Figure S4) in the 'joint evolution scenario', showing that different relationships can emerge, depending on whether traits are allowed to co-evolve or not.

We also briefly reviewed the wide range of taxa where sexual reproduction appears to be linked with dispersal and/or dormancy. We typically reported within-species patterns, i.e. different fates or morphologies of sexual vs. asexual progeny within a species. Earlier studies linking dispersal with dormancy report mixed covariation patterns (Buoro and Carlson 2014), with the majority of studies interpreting the question in an across-species or across-population context. A recent study investigated the correlation of a proxy of dispersal ability (time it takes for a seed to fall in an experimental setting) and dormancy (relative germination rates) in wind-pollinated African Asteraceae species, and found a pattern suggestive of trade-offs at the individual level, but this was restricted to seed-heteromorphic species; the pattern becomes very mixed at population level which then becomes, as a whole, replaced with negative correlations at a species level (de Waal et al. 2015) (see also (Rees 1993)). It appears worth asking, in future studies, whether trade-offs might be responsible for cases where mothers diversify their offspring as dispersal or dormancy specialists, while within-species 'escape syndromes' might exist when one phenotype can efficiently perform multiple escape routes simultaneously. Our results, as a whole, emphasize the message (Buoro and Carlson 2014; de Waal et al. 2015) that it is important to be explicit about the biological scale at which the question is posed, and remind us that sex, too, can be an escape route from a situation where current performance is suboptimal.

Acknowledgements

We thank Isobel Booksmythe for helpful discussion, Anaïs Tilquin for inspiration with the Haiku and the Centre of Excellence in Biological Interactions (Academy of Finland) and the Swiss National Science Foundation for funding.

Data accessibility

The code for this project is available on GitHub: https://github.com/nigerb/CovaCode

Authors' contributions

NG and HK conceived the study; NG and HK constructed the model and analysed the data NG HK wrote and edited the manuscript; all authors revised the manuscript and gave final approval for publication.

Appendix Chapter V

Figure S1 Overview of the 2 x 2 x 2 x 2 scenarios. The same scenarios as in the 'Mother decides' branch are applied to the 'offspring decides' branch, but not shown here due to lack of space. Results for the scenarios are reported in different figures depending on the coloration: Figure 3 shows the results for the scenarios shaded in green, Figure 6 (blue), and the results for scenarios shaded in gray are reported in the supplementary material.

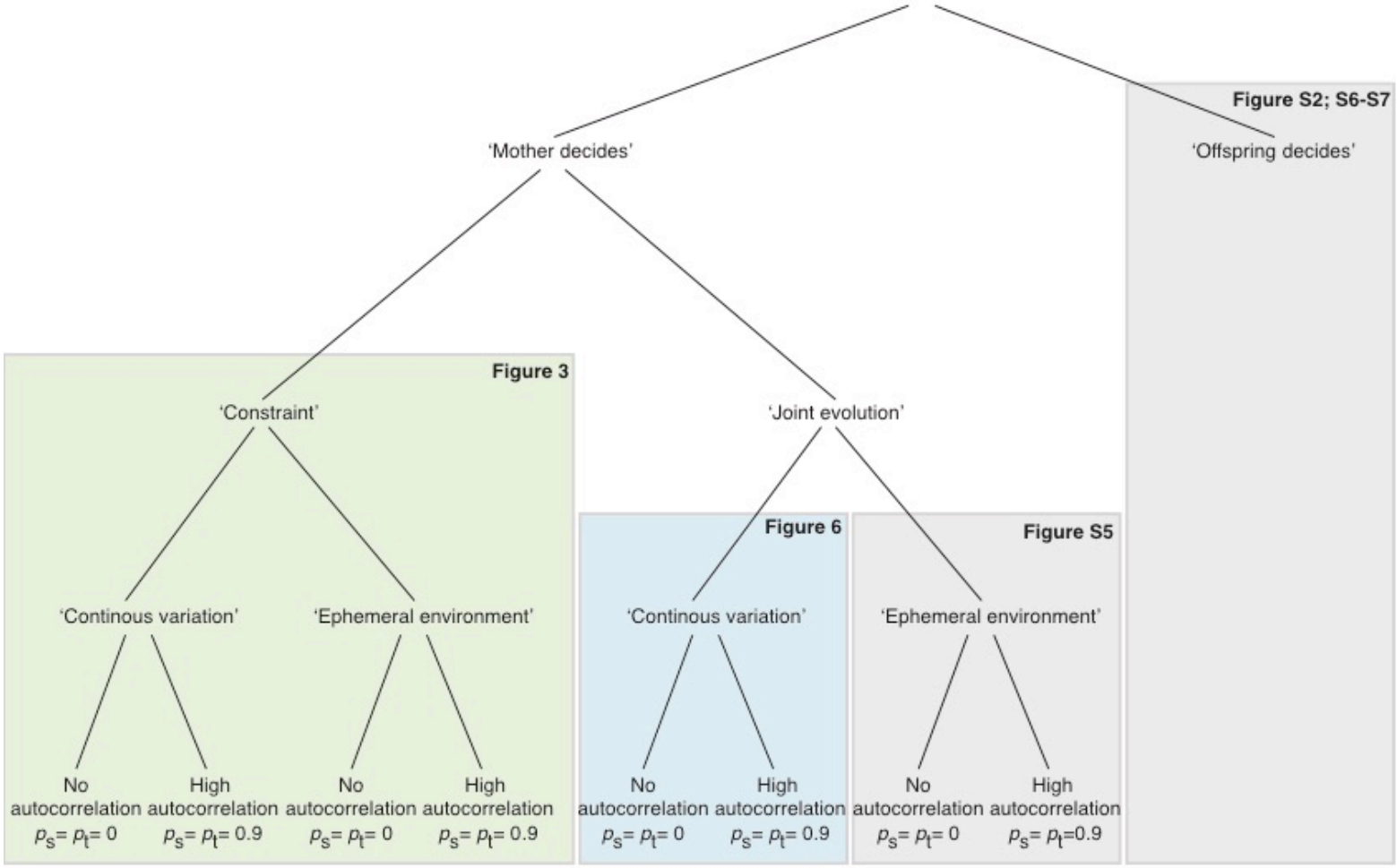


Figure S2 Same as Figure 3 but for the 'offspring decides scenario'. The rate displayed on the y axis was varied for the following values [0.001, 0.002, 0.004, 0.01, 0.0215, 0.046, 0.1, 0.215, 0.46, 1] and the value on the x axis for [0.001, 0.002,0.006, 0.013, 0.032, 0.075, 0.178, 0.422, 1].

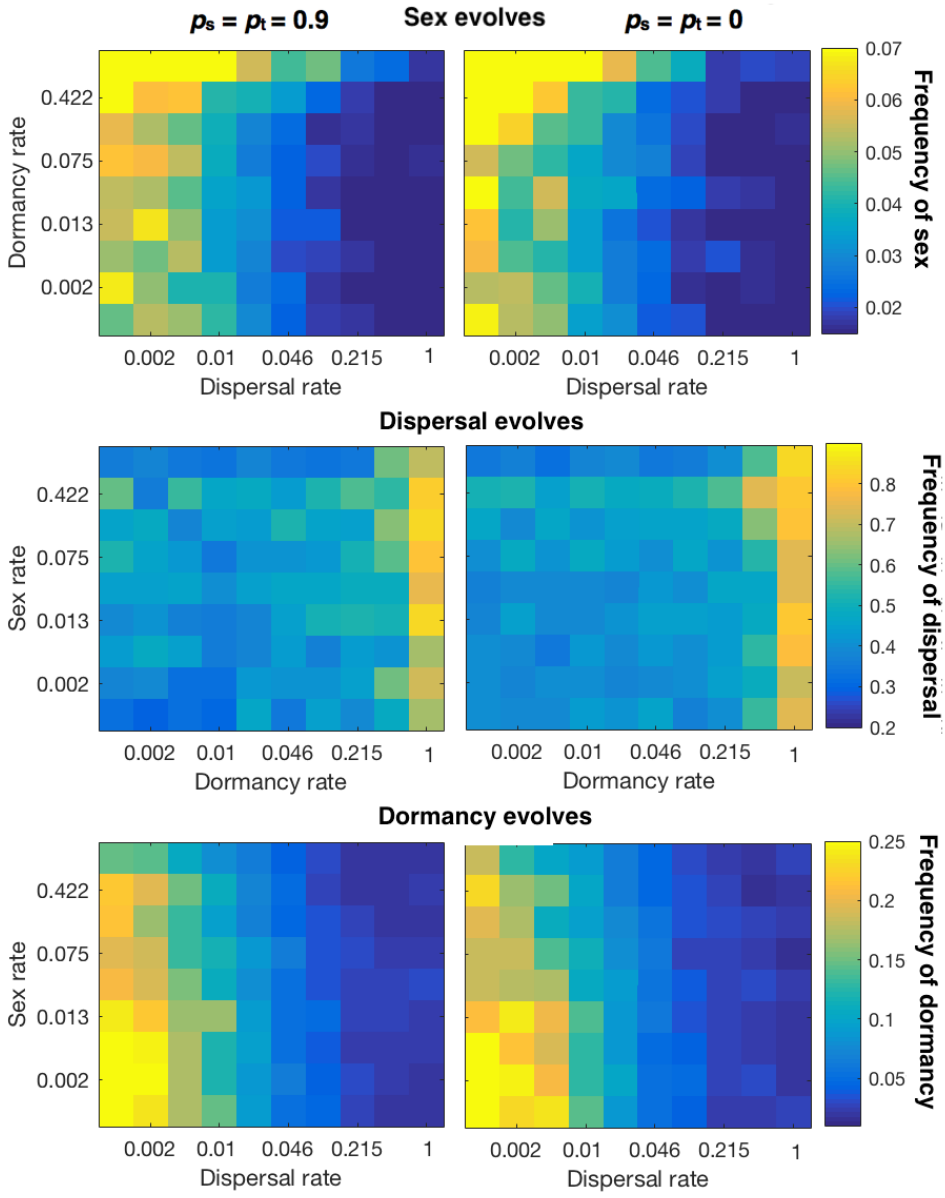


Figure S3 Same data as in Figure 4 but the coloration indicates varying the cost of sex α.

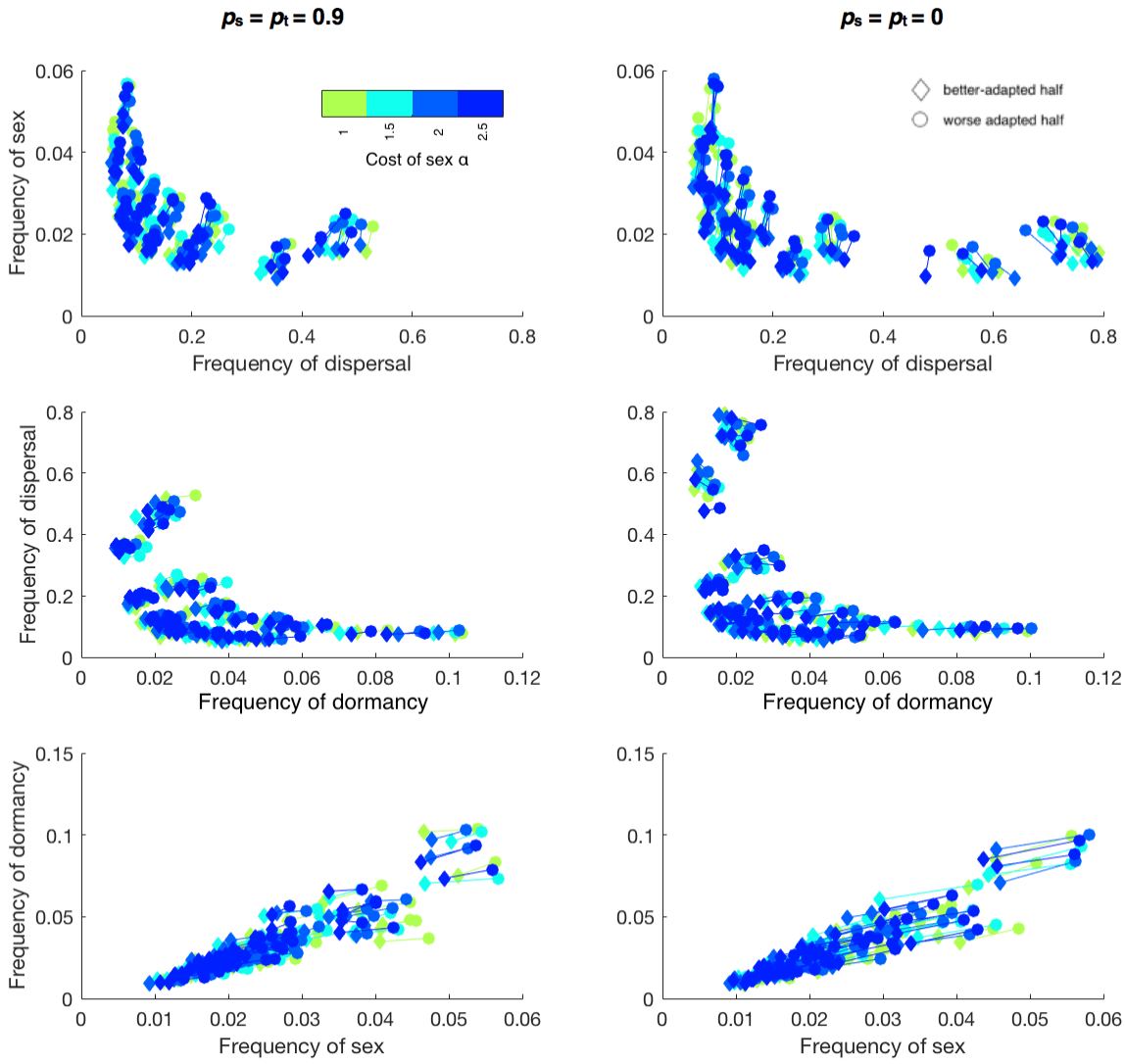


Figure S4 Same data as in Figure 4 but the coloration indicates varying the cost of dormancy τ.

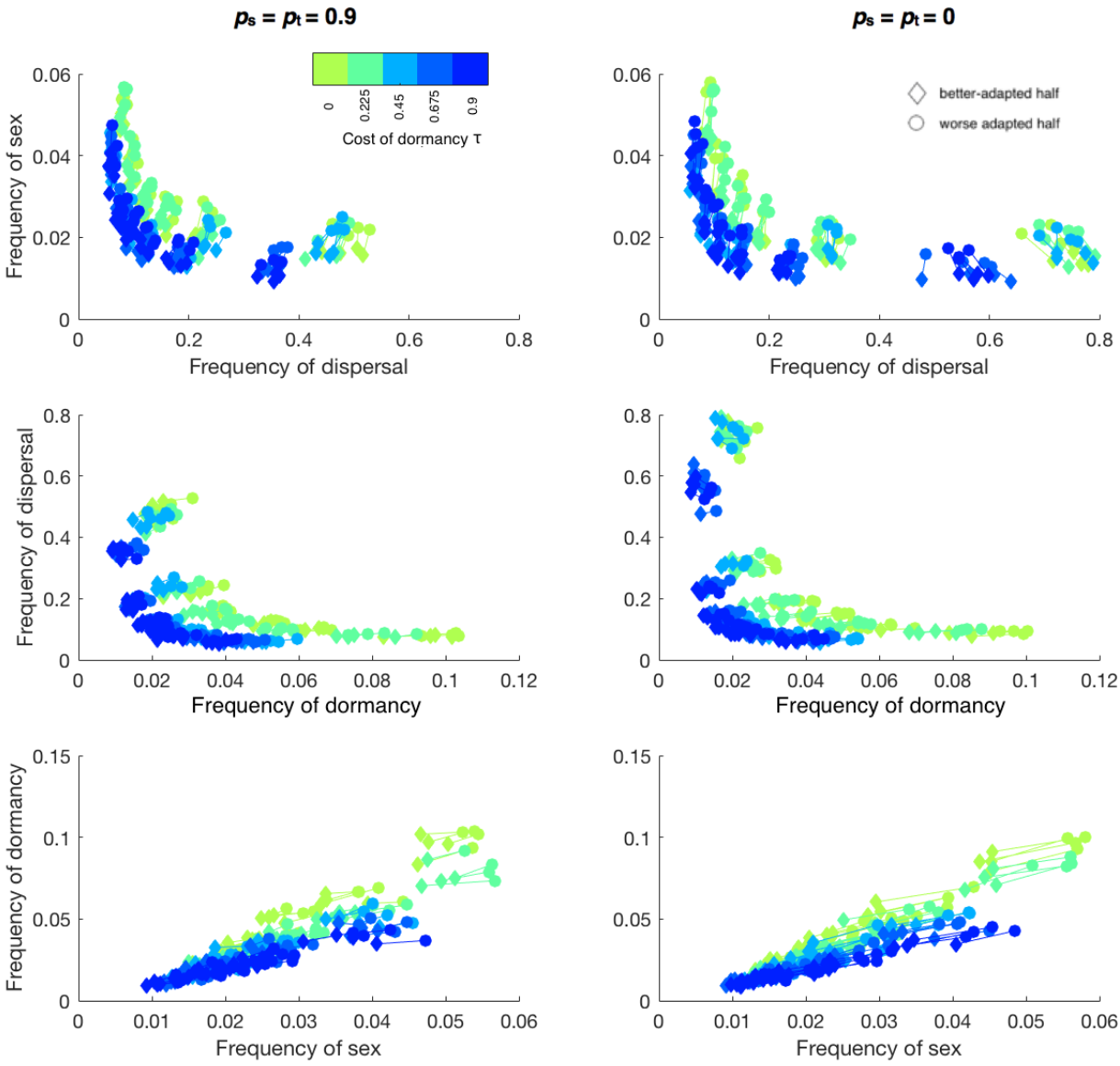


Figure S5 Same parameters as in Figure 4 but for the 'ephemeral environment scenario'.

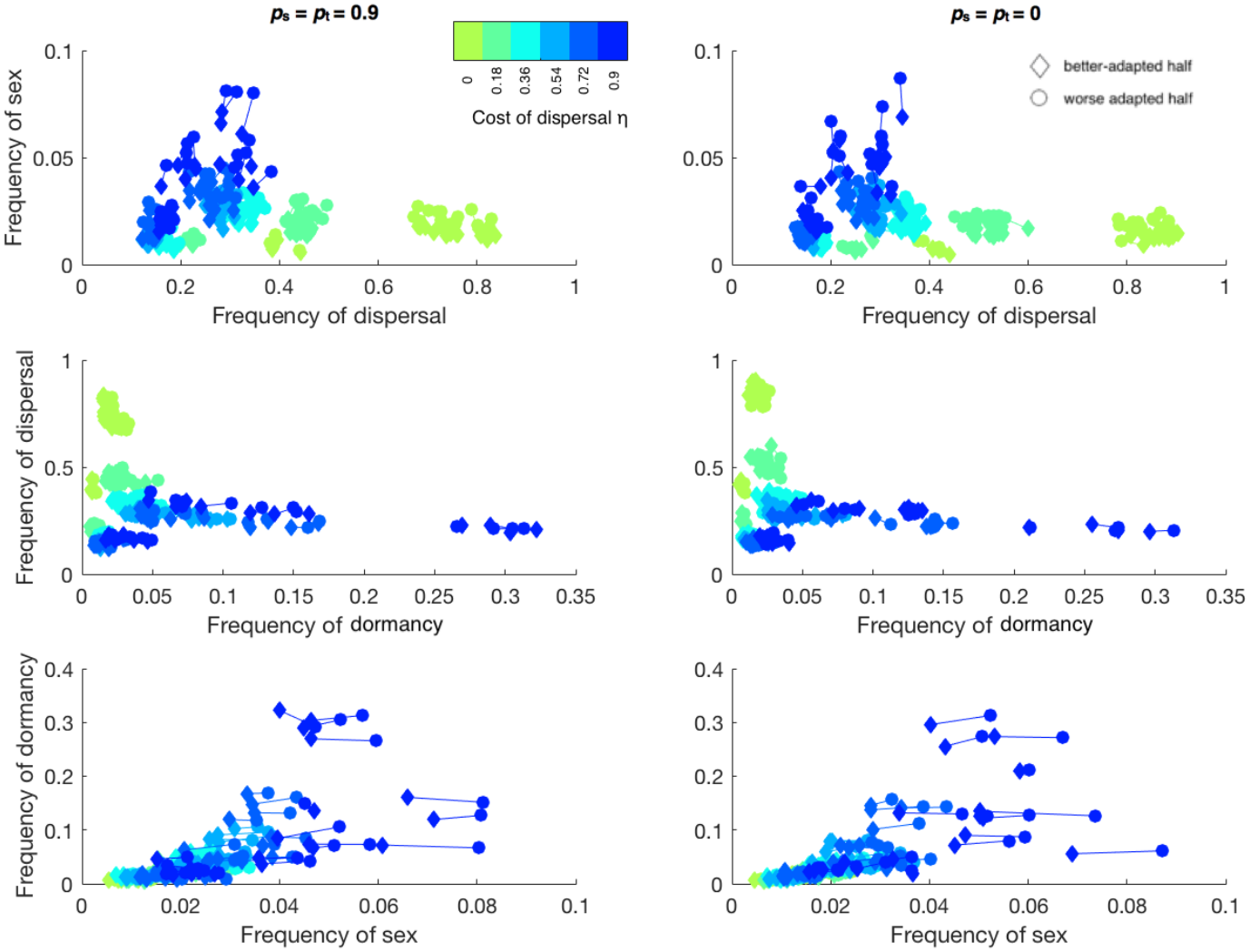


Figure S6 Same parameters as in Figure 4 but for the 'offspring decides' scenario.

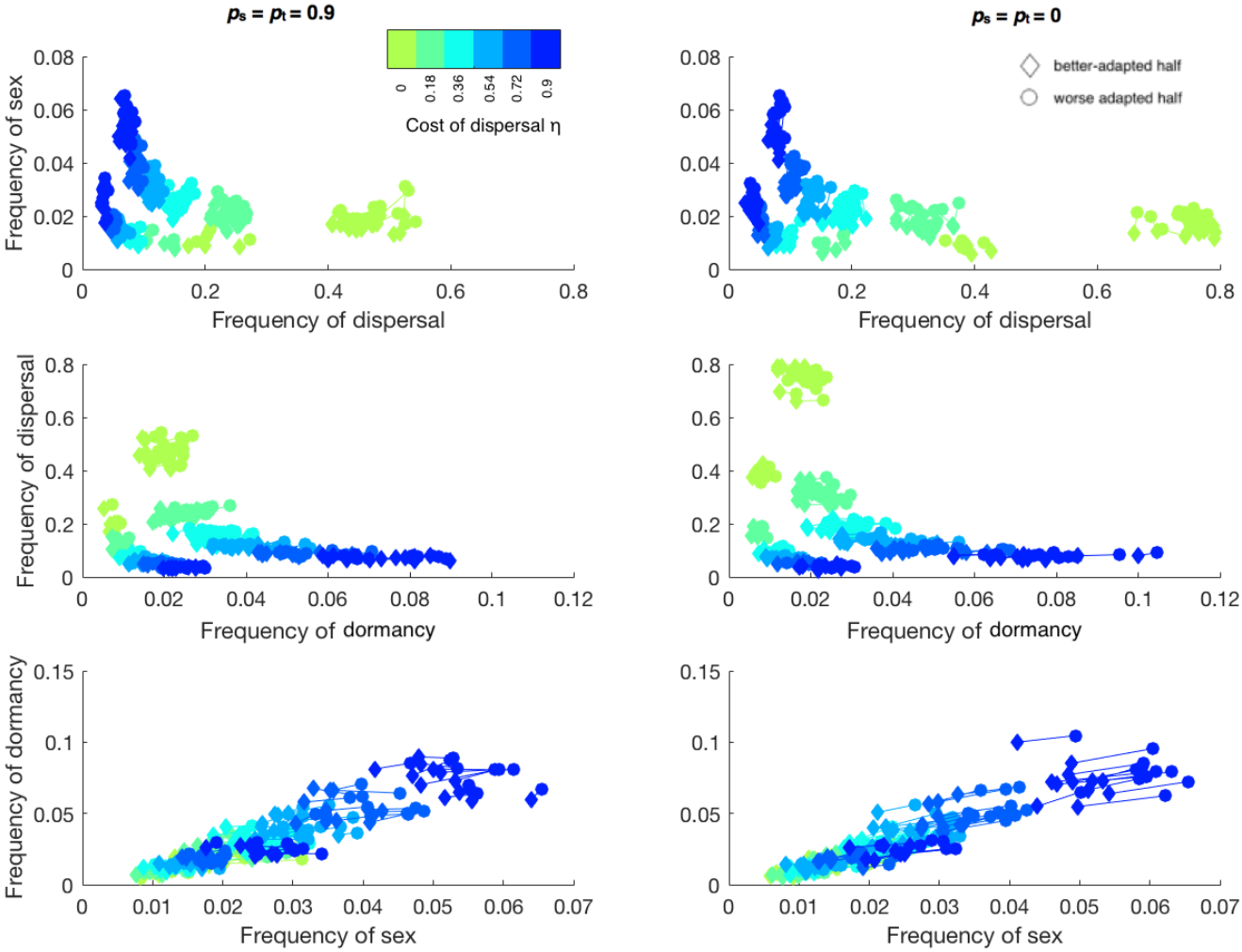


Figure S7 Same parameters as in Figure 4 but for the 'offspring decides ' and the 'ephemeral environment scenario'.

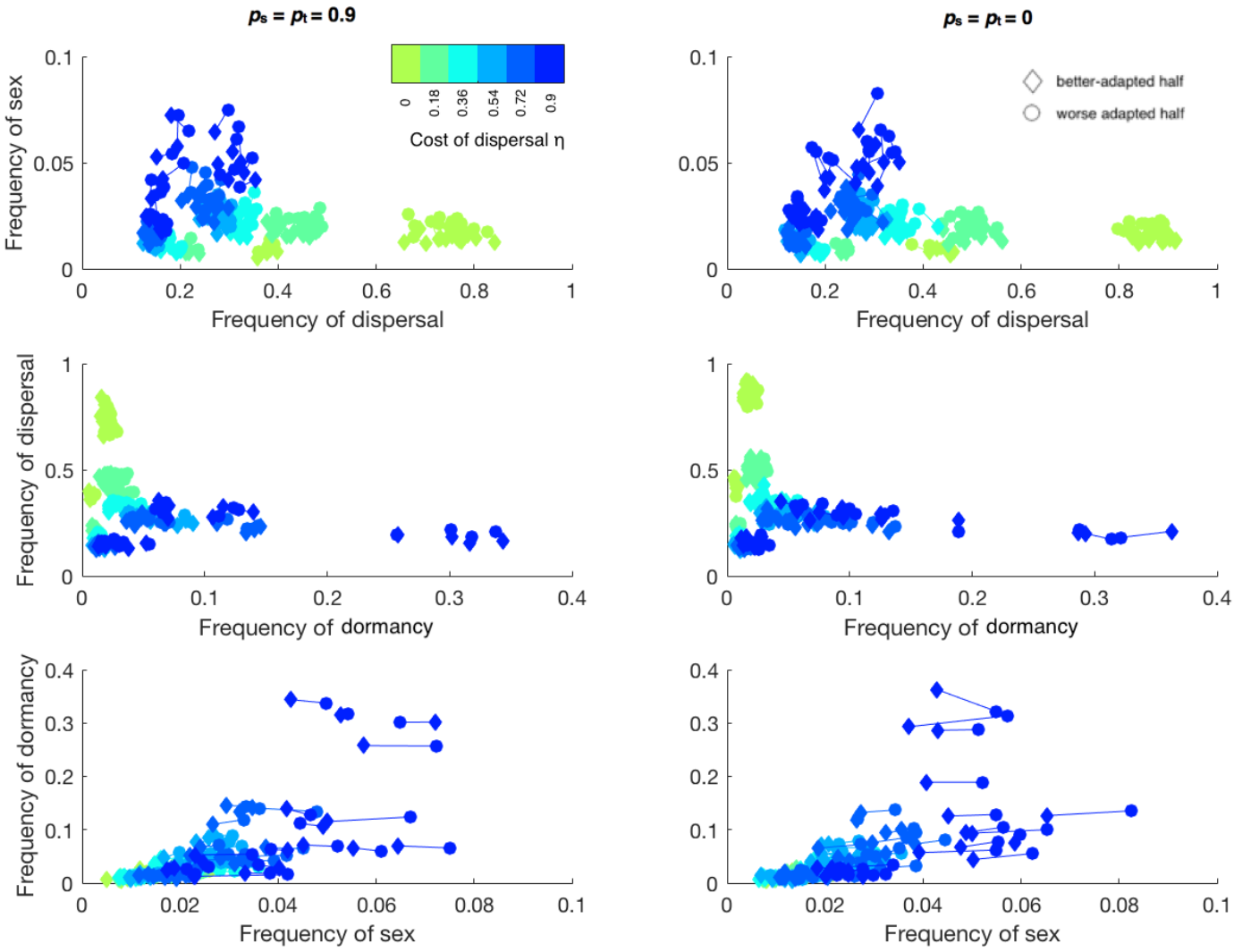


Figure S8 Same data as in Figure S6 coloration according to the cost of dormancy.

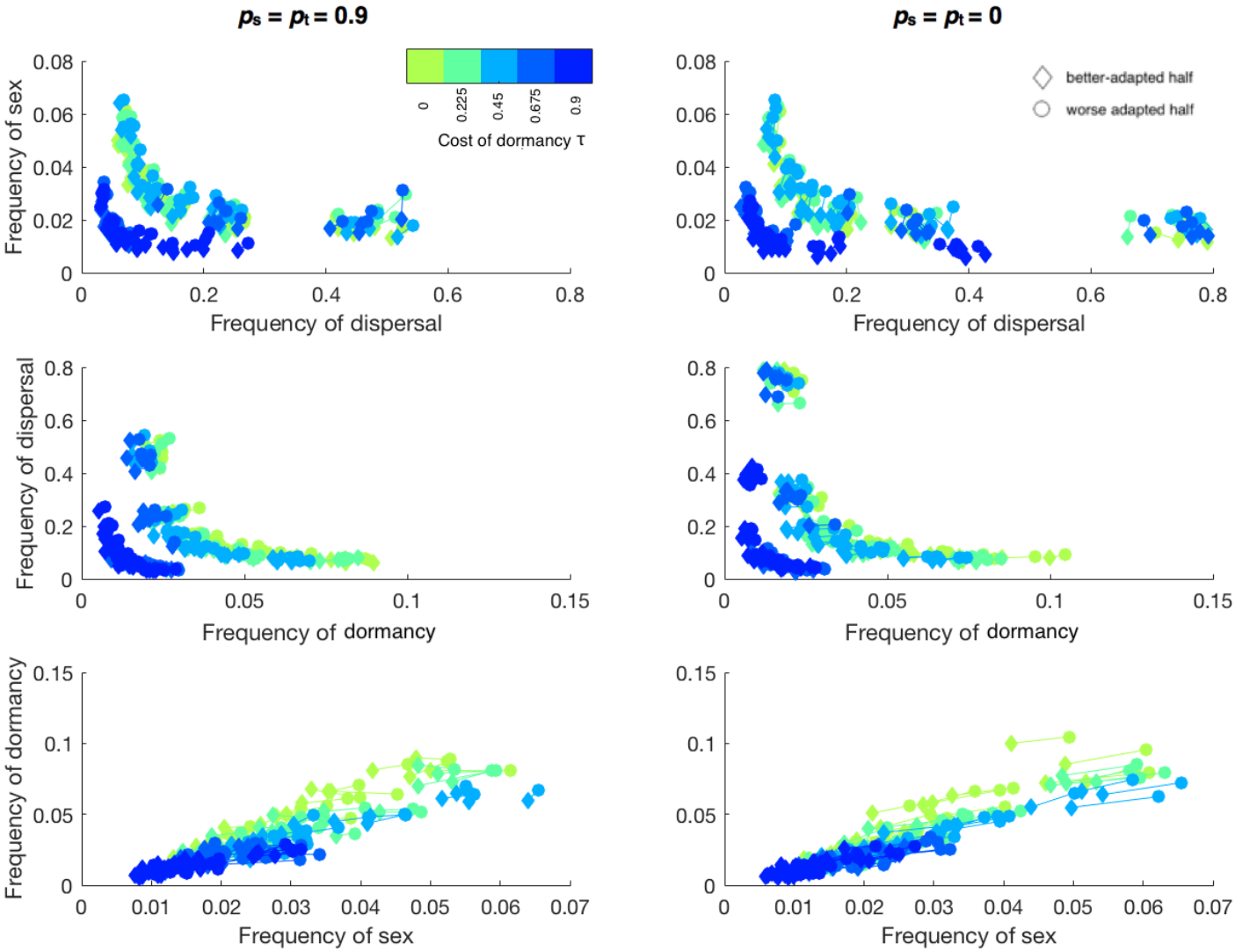


Figure S9 Same as Figure 3 but for the 'ephemeral environment scenario'. Regions where the population went extinct (in all 10 runs) are displayed in gray.

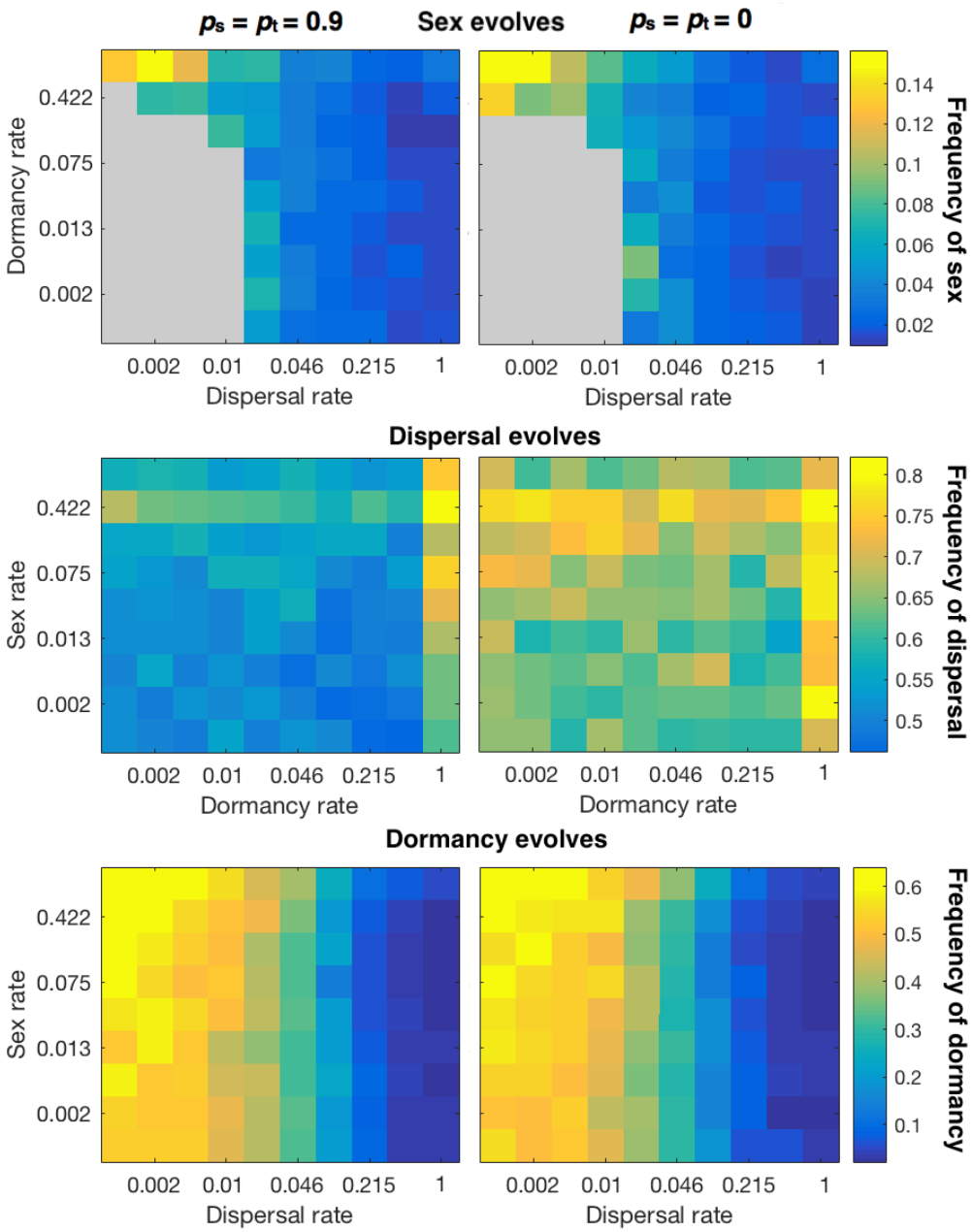
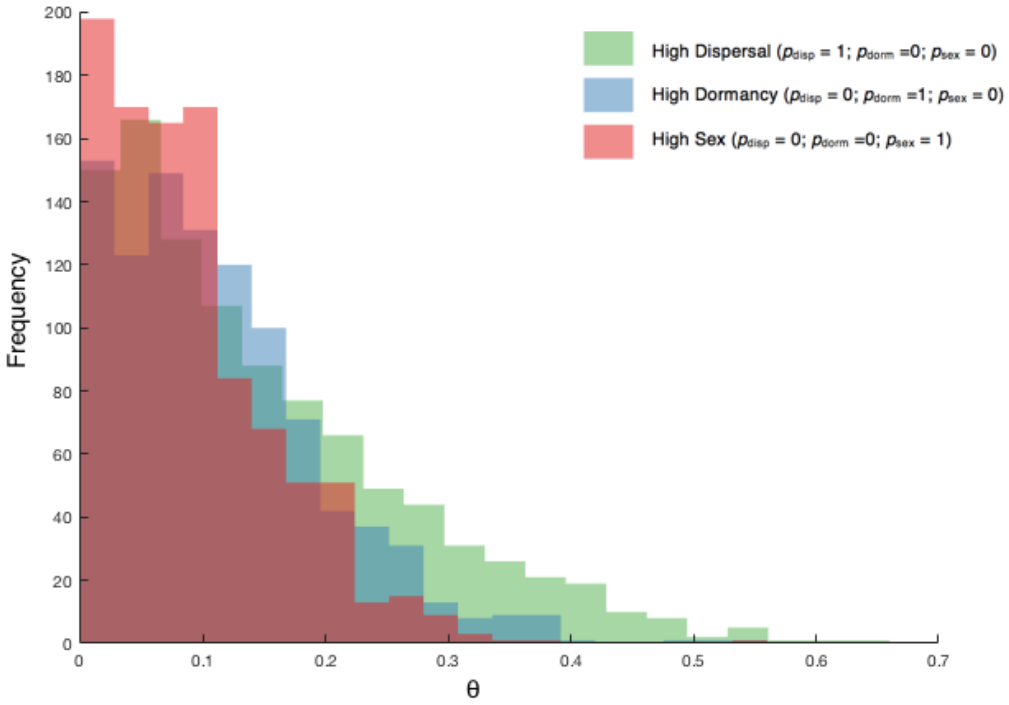


Figure S10 Histogram of θ in a population with high dispersal and no sex and dormancy (green), high dormancy and no sex and dispersal (blue) and a population with high sex and no dormancy and dispersal (red). There is a bigger variation of θ in populations with high dispersal compared to populations with high sex or dormancy. $p_s = p_t = 0.9$, $\tau = \eta = 0.1$, $\alpha = 2$, $w = 0.8$, $D = 1$ and $f = 0$.



CHAPTER VI

Sexual Conflict and The Evolution of Asexuality at Low Population Densities

Asex, f-sex, sex

Males want to mate, females don't

Density matters

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Abstract

Theories for the evolution of sex rarely include facultatively sexual reproduction. Sexual harassment by males is an underappreciated factor: it should at first sight increase the relative advantage of asexual reproduction by increasing the cost of sex. However, if the same females can perform either sexual or asexual life-cycles, then females trying to reproduce asexually may not escape harassment. If resisting male harassment is costly, it might be beneficial for a female to accept a mating and undertake a sexual life-cycle rather than 'insist' on an asexual one. We investigate the effects of sexual harassment on the maintenance of sex under different population densities. Our model shows that resisting matings pays off at low population densities, which leads to the complete extinction of males, and thus to the evolution of completely asexual populations. Facultative sex persists in a narrow range of slightly higher densities. At high densities, selection favours giving up resisting male mating attempts and thus sexual reproduction takes over. These interactions between the outcomes of sexual conflict and population density suggest an explanation for the rarity of facultative sex and also patterns of geographical parthenogenesis, where marginal environments with potentially low densities are associated with asexuality

Introduction

Sex is considered paradoxical because of several known costs of sexual compared to asexual reproduction. These costs include time and energy spent while mating or finding a partner and — in those cases where sex implies a male-female polymorphism — the demographic costs of producing males (Lehtonen et al. 2012). Thus everything else being equal, parthenogenetic females should outcompete sexual lineages because they avoid paying such costs.

Males can also have additional direct negative effects on female fitness, which adds to the twofold costs of producing males (Kokko and Rankin 2007). One example of such a negative effect is sexual harassment by males. Males of many species harass females to achieve copulations (Rowe et al. 1994). Females that try to resist such harassment suffer fecundity or survival costs in a wide range of systems (Shine et al. 2000, Rönn et al. 2006; Gosden and Svensson 2009). For example, female guppies move to habitats of higher predation risk to avoid male harassment (Darden and Croft 2008). Density often plays a role: in high density feral sheep populations, female mortality peaks in summer — despite high resource availability — due to male harassment (Réale et al. 2011). In damselflies, Cordero (Rivera and Andrés 2002) has shown that at high densities more matings were forced compared to low density populations, and that at extreme population density harassment by males can hinder oviposition.

If switching to asexual reproduction is an option, harassment by males should at first sight increase its relative advantage, as the cost of sex is increased. However, this assumes that only sexual females suffer the costs of harassment. This is not necessarily the case (Dagg 2006), reflecting the general principle that any evaluation of the cost of sex should be specific about whether one is comparing the performance of asexual and sexual females within a single population, or growth rates of separate lineages (Lehtonen et al. 2012). If males harass females irrespective of their type and the competition of reproductive types occurs within a population, then parthenogenetic lineages might not manage to escape the costs of such male harassment. It has even been suggested that males should disproportionately target parthenogenetic females with their harassment to maintain sex in a 'spiteful' way (Dagg 2006), but see (Rankin 2008) for problems with this argument.

That male behaviour can have a strong effect on the likelihood of observing sexual reproduction becomes arguably more plausible in the case of facultative sex, where the same organism can perform either sexual or asexual life-cycles (Hartfield 2016). Which one is employed at any given time can depend on environmental conditions, competition, and also – interestingly for our topic – on the availability of males. It is increasingly understood that facultative sex is more than an oddity: rather, it is a fundamental feature of 'early' sex, i.e. that of microbial eukaryotes (Speijer et al. 2015). Facultative sex also occurs in multicellular organisms as diverse as *Daphnia* (Decaestecker et al. 2009), aphids (Simon et al. 2002), Coleoptera (Pollock and Normark 2002), stick insects (Burke et al. 2015), rotifers (Aparici et al. 1998), and many plants (Bengtsson and Ceplitis 2000). Theoretically, facultative sex appears to be a superior solution to either asexuality or obligate sex: it offers much of the genetic advantages of sexual reproduction (e.g. increasing genetic diversity, avoidance of clonal interference, purging deleterious mutations) with a dramatic reduction of the frequency with which costs have to be paid (D'Souza and Michiels 2010), see also (Green and Noakes 1995).

Facultative sex, whenever it associates with a male female polymorphism, has the interesting feature that male fitness improves if as many females as possible undergo sexual life-cycles, but this is not necessarily the case for females (Kawatsu 2013b). This offers the possibility of an interesting demographic feedback because of three interacting factors. First, male harassment, if costly enough, might make it beneficial for a female to accept opting for a sexual life-cycle over an asexual one (this is akin to the argument of 'convenience polyandry' (Griffiths et al. 2012), according to which females might accept costly multiple matings, if the costs of resisting would be higher still). Second, the fitness consequences depend on how often females encounter males. Third, asexual reproduction typically does not produce males. It is also clear that there is potential for the total population density to impact the outcome: in sparse populations, encounters between potential mates happen less often. Sexual conflict and asymmetric evolution of sexual harassment between sexual and parthenogenetic lineages have been considered previously (Dagg 2006, Rankin 2008), but there is limited theoretical work on this evolutionary process regarding the maintenance of sex.

Recent theoretical work has focused on the coevolution of male harassment and females' ability to resist it or its consequences. Kawatsu (Kawatsu 2013*a*) has highlighted the possibility that parthenogens encounter males less often than sexual females. As a consequence, asexuals are hypothesized to suffer higher costs when they subsequently encounter males, because they lack as much coevolutionary experience with males as sexual females have in their evolutionary past (Kawatsu 2013*a*). This can prevent invasions by parthenogens and lead to separate distributions of sexual and parthenogenetic lineages (Kawatsu 2013*a*). Further work has refined these ideas with considerations where the same female can reproduce asexually or sexually depending on an interaction of her resistance and male coerciveness (Kawatsu 2013*b*), including an exploration of the indirect sons' effect where females that resist males less, and consequently reproduce sexually more often, may benefit by producing more coercive (and thus more successful) males (Kawatsu 2015).

Our aim here is to extend previous theory by an explicit look at the role of population density. Feedbacks between population density and sexually antagonistic male-female interactions could play a role in explaining patterns of geographic parthenogenesis, i.e. the finding that the absence of males, as a derived trait, is often associated with marginal environments that are potentially of low population density (Vrijenhoek and Parker 2009). In plants this is often considered to support ideas of reproductive assurance when mate availability is low (Hörandl 2009, Cosendai et al. 2013). Here we highlight the flipside of the coin: male encounters can be harmful, yet resisting them might only pay off sufficiently for females if mate encounters are not too frequent in the first place. If they are frequent, 'mating for convenience' becomes the favoured option, an argument similar to that of 'convenience polyandry', where females mate multiply to avoid the costs of coercive behaviour by males (Griffiths et al. 2012). The frequency of mate encounters depends not only on population density as a whole, but also on how often (other) females produce males, which in turn depends on the population-wide rates of sexual vs. asexual reproduction.

This idea leads to an intriguing possibility of positive feedback in the following sense: In relatively sparse populations, females encounter males at a low rate, and female resistance might be selectively favoured as it allows females to remain virgin for longer (a state in which parthenogenetic reproduction is possible, given a facultatively sexual species). Assuming that parthenogenesis produces female-only offspring, the effect of longer virginity is to reduce male densities relative to female densities in the next generation, which makes resist-

ing females even more successful at avoiding males. Thus, from low enough initial population densities, such feedback might lead to the extinction of males and the population has become *de facto* asexual. By contrast, in denser populations resistance might not pay off to begin with, as the high male encounter rate makes it futile for females to resist; if non-resisting types then take over, the end result is a *de facto* sexually reproducing species as the time spent as a virgin remains very low.

Methods

Model description

Our model considers a species in which females are, at least ancestrally, capable of both asexual and sexual reproduction. Virgins reproduce asexually, producing only female offspring. After mating females reproduce sexually and produce male and female offspring at a 1:1 ratio. Males are assumed capable of forcing females to mate with them, but with their efficiency depending on female resistance. After a successful mating, a female reproduces sexually for the rest of her life. In the population, there are four types of individuals with haploid genetics. Females express a resistance allele such that *r* females never resist a male mating attempt, while *R* females are partially successful with their resistance attempts and are thus likely to remain asexual for longer. The strength of the resistance is denoted θ, such that resisting females only mate with every θth male on average (i.e. their mating rate is obtained by dividing the non-resisters' mating rate by θ). When $\theta = 1$, *R* and *r* females behave in the same way. Values for $\theta < 1$ are biologically not relevant and thus not considered in our model. Each male carries a copy of the *r* or the *R* allele but these are not expressed. The total density of the population, *D,* is the total number of males, *M*, plus the total number of females, *F*, in the population.

We consider discrete generations, but we model within-season dynamics in continuous time. Females and males continually enter the population while the season lasts; we thus do not model any seasonal fluctuations of females or males arriving or dying, which we justify by the fact that the availability of *r* vs. *R* carrying males should not be seasonally dependent for females if these alleles are not expressed in males. Females' death rate needs to be explicitly modelled, however, as we need to contrast the lifespans of *r* and *R* females as well as quantify the proportion of it that is spent in a virgin state. We assume that females produce eggs at a continuous rate while alive. Each female may go through one transition from being unmated (a virgin) to being mated, and the rate at which the transition happens depends both on male density *M* (mate encounters per female happen at a rate that is proportional to the total density of males; we assume a proportionality constant of 1) and the female's strength of resistance behaviour θ (see table S1 for model parameters). For each generation we derive the expected number of eggs produced by virgins and non-virgins and the genotypes of these eggs, which then determines the frequencies of *r* and *R* males and females in the next generation.

Analytical calculations

To derive the number of eggs of each type produced we first derive the expected lifespans as virgins and non-virgins for each type of female. Each female begins her life as a virgin, and this state can end either by mating or by death. As a null model for mate encounter rates one can take the 'ideal gas approximation' (see (Hutchinson and Waser 2007)), according to which a density of F females and M males leads to FM mate encounters per time unit. Here our scaling of densities and time units are chosen such that a density $F = M = 1$ will lead to one observed mate encounter per time unit. As this time unit is also the mean lifespan of a non-resisting female (see below), $F = M = 1$ is a density where females only meet on average one potential mate in a lifetime; most natural populations probably occur at higher densities, which we also consider.

Doubling the density of both males and females will, in the null model of 'ideal gas' type, lead to a twofold per capita rate for individuals of either sex, or a fourfold observed number of mate encounters in the population as a whole. There are also situations where the kinetics of encounters follows other exponents than what is suggested above; for example, if encounters take time to complete, then the per capita encounter rate grows less than linearly (also expressable as the total number of encounters growing subquadratically (Nicolis et al. 2005). We model this with an exponent β, presenting results based on the null model ($\beta = 1$) in the main text and results for $\beta \neq 1$ in the supplementary material.

We assume that resisting a male is costly, thus the mortality of resisting females is modelled as

$$\mu_R = 1 + \alpha(\theta - 1)M^{\beta}$$

whereas non-resisting females have a mortality of $\mu_r = 1$ (as for these females θ is replaced by 1), which implies an expected lifespan of 1 unit of time. β is a parameter for the strength of the costs of resisting a male. Since $\alpha > 0$ and $\theta > 1$, resisting females are assumed to have shorter lifespans (below 1) than non-resisting females. The excess mortality term increases linearly with the number of mate encounters M^{β}, as more frequent encounters imply that the female needs to activate her resistance behaviours more often.

Our computations take advantage of the fact that if two events happen at a rate υ and μ and either can end the state that an individual finds itself in, then the expected time the individual spends in this state is $1/(\upsilon+\mu)$. Virginity can end by mating or death. The rate of mating is $\frac{M^{\beta}}{1}$ for r females, as they mate with every male they encounter, and $\frac{M^{\beta}}{\theta}$ for R females. The expected time a resisting (R) female stays virgin, denoted T_{virgin_R}, is impacted by her mating rate $\frac{M^{\beta}}{\theta}$ as well as her mortality μ_R:

$$T_{\text{virgin}_R} = \frac{1}{\frac{M^{\beta}}{\theta} + \mu_R}$$

For non-resisting females, the equivalent expression is

$$T_{\text{virgin}_r} = \frac{1}{M^{\beta} + \mu_r}$$

The expected time spent as a non-virgin female is calculated as the probability that mating rather than death ends a female's virgin time (as dead virgins never contribute to non-virgin time), multiplied by the expected time alive after the first mating. Again, we take advantage of mathematical knowledge that if two event types 'compete' to end a state and they occur at rates υ and μ, then the probability of υ being the ending transition is υ/(υ+μ). Thus, the expected time after mating becomes

$$\mathrm{T}_{\mathrm{mated}_R} = \left(\frac{\frac{\mathrm{M}^{\beta}}{\theta}}{\frac{\mathrm{M}^{\beta}}{\theta} + \mu_R}\right)\frac{1}{\mu_R}$$

$$\mathrm{T}_{\mathrm{mated}_r} = \left(\frac{\mathrm{M}^{\beta}}{\mathrm{M}^{\beta} + \mu_r}\right)\frac{1}{\mu_r}$$

for the two female types. Note here, that the expected time females of either type spend in a certain state can be calculated, even if their frequency in the population is negligibly low.

Population dynamics

We assume that egg production occurs at a constant rate, thus egg production per generation is proportional to the time females spend in each of these states. Note that sexual females of either genotype can produce *r* as well as *R* eggs, because the sire of their offspring may be of either genotype. During the times of virginity, however, each type of female produces only its own genotype.

With a given initial genotype frequency and an initial sex ratio we can now calculate $E_{♀_r}$, the number of female eggs that are *r* in the next generation: these are all the eggs produced by *r* females while being virgin, half the offspring (i.e. all the females) from an *r* × *r* mating, a quarter of the offspring (half of all the females) from an *r* × *R* mating where the 2nd letter denotes the male, and a quarter of the offspring from an *R* × *r* mating:

$$E_{♀_r} = F_r \mathrm{T}_{\mathrm{virgin}_r} + \frac{1}{2}F_r \mathrm{T}_{\mathrm{mated}_r}(1-\rho) + \frac{1}{4}F_r \mathrm{T}_{\mathrm{mated}_r}\rho + \frac{1}{4}F_R \mathrm{T}_{\mathrm{mated}_R}(1-\rho)$$

Here, ρ refers to the proportion of *R* males among the potential sires. As we assume no life history or behavioural differences between male genotypes, this proportion is simply

$$\rho = \frac{M_R}{(M_R + M_r)}$$

The same way the number of *R* female eggs produced can be calculated:

$$E_{♀_R} = F_R T_{virgin_R} + \frac{1}{2}F_R T_{mated_R}\rho + \frac{1}{4}F_R T_{mated_R}(1-\rho) + \frac{1}{4}F_r T_{mated_r}\rho$$

Males are only produced when the females have already mated. The number of male *r* eggs that are produced are calculated as the sum of *r* males produced by *r* females and *r* males that are produced by *R* females:

$$E_{♂r} = \frac{1}{2} F_r \mathrm{T}_{\mathrm{mated}_r}(1-\rho) + \frac{1}{4} F_r \mathrm{T}_{\mathrm{mated}_r}\rho + \frac{1}{4} F_R \mathrm{T}_{\mathrm{mated}_R}(1-\rho)$$

and similarly for the number of *R* male eggs that are produced:

$$\mathrm{E}_{♂R} = \frac{1}{2} F_R \mathrm{T}_{\mathrm{mated}_\mathrm{R}}\rho + \frac{1}{4} F_R \mathrm{T}_{\mathrm{mated}_\mathrm{R}}(1-\rho) + \frac{1}{4} F_r \mathrm{T}_{\mathrm{mated}_r}\rho$$

When no males are present there are no changes in the composition in the population as *r* females can only produce *r* females, *R* females can only produce *R* females, and there are no lifespan differences between *r* and *R* females if there are no males harassing them.

The above equations complete the description of the within-generation dynamics. At the end of every generation, the equations above give the new genotype frequencies. We then normalise them to sum up to the assumed population density, *D*, which allows us to track the genetic changes under a variety of low or high density scenarios. To see how the dynamics change when population densities change we tracked the within-generation dynamics starting with a given genotype frequency, for different population densities *D*. Simulations were initiated with half of the population being male, but the proportion quickly changes as a result of male production in the first generation.

Results

Although all populations in all generations are technically facultatively sexual, they can become functionally sexual (if the virginity period of females remains very short) or functionally asexual (if males go extinct). It is also possible that the population remains in a state where virgin females perform a significant fraction of reproduction, but not all of it. In principle there are six possible outcomes: Males and females of one type (either *r* or *R*) survive, only females of either type persist, females of both types coexist, or males and females of both types coexist. The realized outcome is dependent on the population density, the initial frequency of the resistant type and the strength and the costs of resistance. We will first present results from isolated population densities that show the dynamics over several generations and then summarise the evolutionary stable outcomes over a density continuum. The code to produce the simulations is provided in the ESM.

At high or intermediate densities, males can exist with either acceptance or resistance behaviours having become fixed in females

At high to intermediate population densities the results are independent of the initial frequency of the resistant type in the population. At intermediate density the resistant *R* genotype becomes fixed, and resistant males and females persist in the population (Figure 1a). Here *r* goes extinct, because at these densities male encounter rate is high enough that *r* females — who do not resist mating attempts — bear the demographic cost of producing males for a larger proportion of their lives (Figure 1a). *R* females have higher mortality, but they spend more of their shorter lives in the virgin state, which as a whole can lead to a sig-

nificant improvement in the number of daughters produced in a lifetime. As the benefits of reproducing asexually outweigh the lifespan cost of resisting mating attempts, *R* females outcompete *r* females. Although the *r* allele is driven to extinction, males are not. This is because *R* females also have some expected duration of sexual reproduction as long as resistance is not assumed to be perfect (our example uses $\theta = 5$), thus a small proportion of *R* males are continually being produced. As a whole, facultative sex can persist indefinitely in populations of intermediate densities (Figure 1a).

When population density is much higher, females of either type spend most of their lifetime reproducing sexually. *R* females cannot delay mating for long, but they also suffer continually the costs of attempting to resist, resulting in a significant shortening of their lifespan compared with *r* females (Figure 1b). These fitness costs lead to the extinction of the *R* allele (Figure 1b); the phenotypic outcome is that females no longer try to resist male mating attempts. When females reproduce mainly sexually (each female's initial period of virginity remains brief), the male densities become high, and this further shortens the virginity period. Given that we assume that females produce a 1:1 sex ratio after mating, we find that the sex ratio of a dense population that is monomorphic for the *r* allele evolves to be close to 1 (Figure 1b). The stable evolutionary endpoints in Figure 1 are independent of the initial genotype frequencies (the figures use an initial frequency 0.5 for *R*).

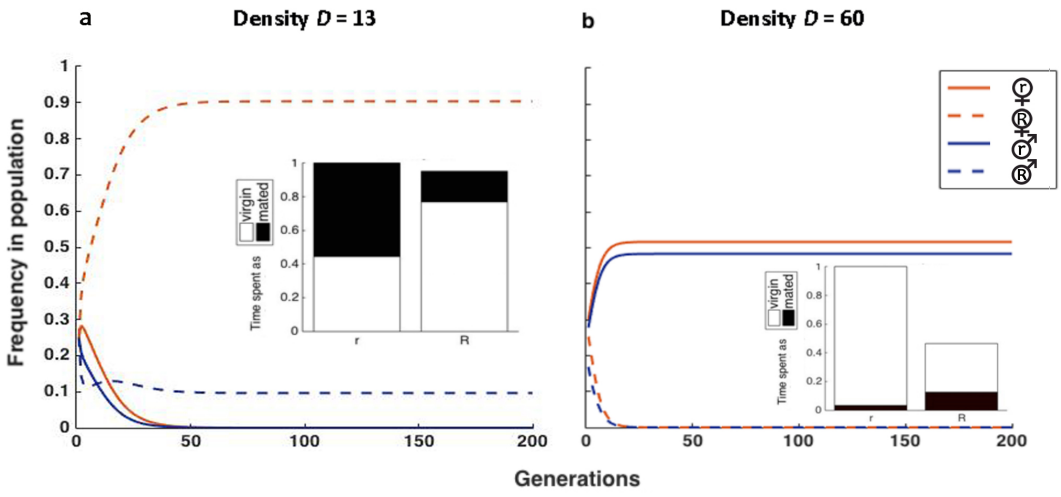


Figure 1 The frequency of each type in the population over 200 generations at two densities (a) *D* = 13 and (b) *D* = 60. The embedded barplots show the expected lifespans of *R* and *r* females and the proportions that they spend in the virgin state at the given population densities at equilibrium. Other parameters: $\theta = 5$, $\alpha = 0.01$, $\beta = 1$, and the initial frequency of *R* is 0.5. The online version is in colour.

At intermediate or low densities, initial frequencies can matter

In between the cases of fixation of either *r* or *R*, as described above, there is a region of density values where the initial frequency of *R* females determines the outcome. When the initial frequency of *R* is low (denoted '*R* invasion'), the dynamics lead to the persistence of *r* males

and females and the *R* genotype goes extinct (Figure 2c). Starting from high initial frequency of *R* (denoted 'r invasion), however, leads to the opposite case where the invasion of *r* fails, and *R* males and females persist at equilibrium (Figure 2d, see also Figure S3).

The initial frequency also influences the outcome at the low end of population densities. Low densities lead to low mate encounter rates, thus females of either type (*r* or *R*) remain virgin for most of their lifetime and consequently reproduce mostly asexually. Few males are produced, which again promotes asexual reproduction by prolonging the likely time a female spends in the virgin state; this is a quick demographic process that makes males go rapidly extinct. Once males are extinct, resistance has become a neutral trait as the absence of males means females never express their resistance alleles. In other words, genotypes *r* and *R* no longer impose mortality differences and both lead to completely asexual reproduction; they are subsequently only subject to genetic drift (which we do not model explicitly). In this process, the type of female that persists in the population — or whether there is coexistence subject to drift of both types — is dependent on the initial frequency of the *R* and *r* individuals (Figure 2a and 2b).

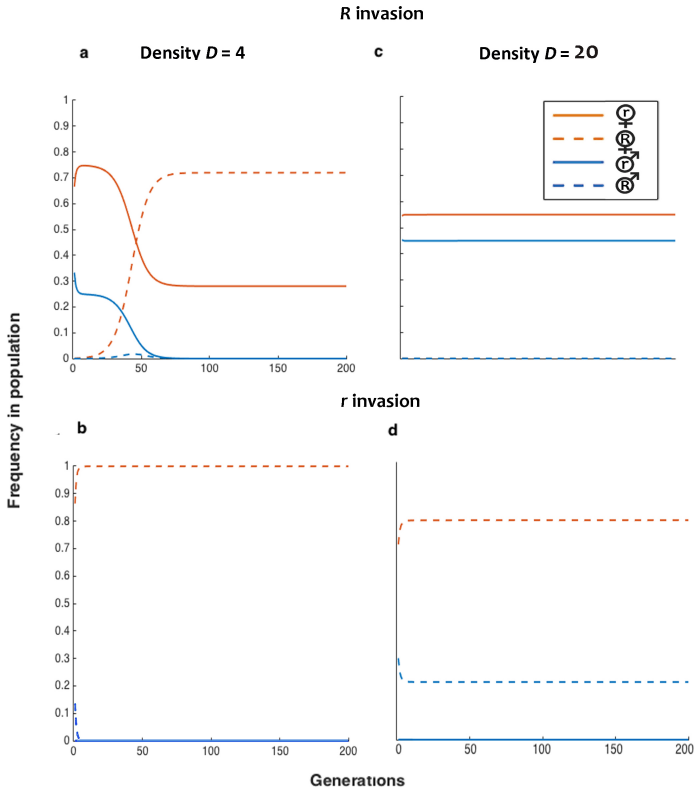


Figure 2 The impact of initial frequency on the evolution of resistance and the demographic consequences. The composition of the population is shown for 200 generations at densities D = 4 (a, b) and D = 20 (c, d), starting from an initial frequency of R of either 0.001 (a, c) or 0.999 (b,d). Other parameters: $\theta = 5$, $\alpha = 0.01$, $\beta = 1$. The online version is in colour.

At very low population density a genotype's initial frequency is correlated with its frequency at the point where males have become extinct. But at somewhat higher population density, the production of males persists as an outcome of relatively frequent successful mating attempts by males of the parental generation; this means *R* is continually selected for and can become fixed even from a very low starting frequency (see Figure 3a).

From no males to facultative sex to a de facto sexually reproducing population

The generality of the results above can be shown with a continuum of densities (Figure 3). At very low densities, males go extinct and resistance becomes a neutral trait, with the outcome simply depending on the initial frequencies in the population (densities below 10 in Figure 3). At somewhat higher densities (above 10 in our example), males can persist, which means that there is always a difference in how *r* and *R* females gain fitness. Females that mate bear the demographic costs of producing males. *R* females remain virgin for longer than *r* females, and thus avoid these costs for longer; but they also pay costs of resisting, the magnitude of which depends on the frequency of males in the population. If the total density remains low (and thus mate encounters remain relatively infrequent), resisting pays off and *r* disappears, but since resisting cannot make a female avoid all matings, males persist.

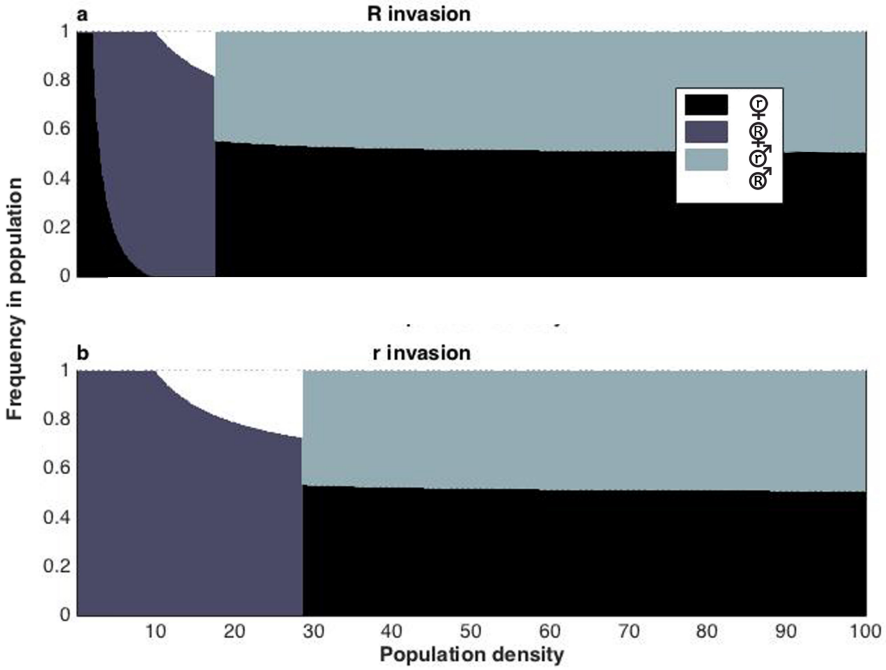


Figure 3 The population composition as a function of population density at stable evolutionary endpoints, (a) for an initial frequency of *R* individuals of 0.001 and (b) for an initial frequency of *R* individuals of 0.999. Other parameters: $\theta = 5$, $\alpha = 0.01$, $\beta = 1$.

When *R* is fixed, male abundance increases with increasing population density, and this case (from density 10 onwards in Figure 3) is the endpoint for a greater range of densities if resistance was initially common (Figure 3b) than if it was initially rare (Figure 3a). This outcome is eventually replaced by a *de facto* sexually reproducing population, with all females accepting going for sexual cycles as soon as they encounter males (*r* fixed), when densities are high enough (Figure 3, right side of either panel). The higher the proportion of *r* individ-

uals in the starting population, the lower is the density at which point the transition to a complete *r* population happens. At what density it pays for females to accept a sexual life-cycle and from which density upwards males can exist, is also dependent on the strength of resistance θ and the costs of resistance α, but the qualitative pattern remains unchanged (i.e., high density promotes sexual reproduction) for alternative values and random combinations of these parameters (see Figures S1–S4).

Variations in other parameters, such as β (Figure S5), the initial frequency of the *R* allele (Figure S3), or the initial sex ratio (Figure S6), have only minor effects. Of these effects, the most interesting one is that when β is low, the density range that permits facultative sex becomes larger (Figure S5). Note that low β introduces a nonlinearity that makes both high and low densities 'resemble' intermediate densities in terms of the mate encounter rate. The biological interpretation is that all else being equal, prolonged mate encounters make facultative sex evolve more easily.

Once sexual reproduction is the norm, the sex ratio approaches 1:1 (right end of Figure 3 and Figure S4); the sex ratio change with population density is non-linear (Figure 3 and FigureS4). Note that we predict mild deviations from 1:1 at the low density end of *de facto* sexuality, and these deviations disappear as we move to the right along the density axis. This occurs because our model still allows for virgins to reproduce for the short amount of time that they spend before their first mate encounter.

Our model can also be used to ask what happens if costs of resistance disappear. In this case, resistance pays off as soon as there are any males in the population. Thus, at low densities, non-resistant females can persist, but at densities that permit males to exist, resistance becomes fixed, also at high population densities. Males can also persist given that a finite θ does not prevent all matings (Figure S7).

We also asked what happens if matings do not involve sexual harassment ($\alpha = 0, \theta = 1$). In this case, only the initial frequency determines which genotype persists. Male extinction in the main model occurs because of a positive feedback where scarcity of mate encounters leads to low production of males, which further reduces mate encounter rates until no males are produced. While strong females resistance widens the range of densities where males go extinct, a narrow range of 'self-extinguishing' male demography (due to the same demographic feedback) persists even if there is no effective resistance by females (Figure S8).

Discussion

Our model investigates sexual conflict of a profound kind: males can only achieve reproductive success via mating, while females in principle have the option to reproduce without the aid of males. This leads to a conflict of interest between males and females, as for the latter, not mating is often desirable. The expected consequence is that it takes effort for females to resist male mating attempts, and here we show a rich set of dynamic outcomes that depend strongly on population density.

Whether or not females evolve to accept 'mating for convenience' can lead to the maintenance of facultative sex at intermediate densities, to complete asexuality at low densities, or to *de facto* sexuality at high densities. Our model thus extends the argument of 'convenience polyandry' (Lee and Hays 2004; Griffiths et al. 2012) to a situation where the choice is not

between mating once or multiply, but between mating at all (once or multiply) or reproducing parthenogenetically.

High population density combined with male harassment leads to the promotion of sexual reproduction and thus the production of males, even when females can resist such harassment. At very low population densities, there is strong enough positive feedback between a female-biased adult sex ratio and the realized time each female spends as a virgin, that males become extinct (at low enough population density this can happen even when females do not resist matings, see Figure S8). Despite the potential for a sexual cycle to still exist, the observed population would thereafter only perform asexual cycles.

When switching to sex is costly, female resistance to mating can maintain parthenogenetic reproduction, as long as the costs of resistance are outweighed by the benefits of asexual reproduction (Arnqvist and Rowe 2013). In the Australian spiny leaf stick insects, for example, switching from asexual to sexual reproduction imposes fitness costs for the female (Burke et al. 2015). Such costs of switching to sexual reproduction and the relative low costs of resistance at low population densities can promote conditions that favour the evolution of parthenogenesis. Note that even though we do not predict that virgin parthenogenesis followed by sexual cycles of mated females should be a common finding (it becomes easily replaced by other options), the important assumption is that there is potential for life histories to be shaped by this option. This appears throughout possible, e.g. in the form of tychoparthenogenesis, which is the rare hatching of unfertilized eggs in a wide range of normally sexual invertebrates (Schwander et al. 2010).

At what densities accepting a mating becomes the favoured option from females, depends on the strength and the costs of resistance and on the initial frequency of the resistant type in the population. This is a typical feature of models with positive feedback (Lehtonen and Kokko 2012): history matters, and in our case this principle manifests itself as phylogenetic inertia at low to intermediate population densities. It is difficult for a *r* population to invade one in which *R* is established (and vice versa). Future work could usefully investigate if this difficulty still persists if resistance was a continuous rather than a discrete trait (see below for further discussion of model limitations).

Sex in most organisms appears obligate, even though facultative sex appears to offer an adaptive solution that maintains most of the benefits while minimizing the costs. Our model suggests a partial solution to this puzzle: as long as males exist, it is in their interest to keep females sexual, and if they possess means to achieve this outcome, it may be too costly for females to resist. Our model therefore produces a large parameter region (at sufficient population densities) where sex predominates. We have not explicitly modelled any subsequent evolutionary phase where a functionally sexual population turns obligately sexual. However, it is conceivable that the ability to perform an asexual life-cycle could be lost in high-density populations, if females rarely manage to express this option. Thus obligate sex could have evolved from facultative sex as a consequence of sexual conflict.

Studies of mate rejection in facultatively sexual organisms are rare (Burke et al. 2015) (Schmit et al. 2013), and it is difficult at present to find studies that would track life events of females living at different densities (there is an understandable tendency for researchers in general to favour studying organisms in locations where they are common). Our model assumes that male harassment is a bigger problem for females at high density; evidence for this is mainly available for obligate sexual species [(Gosden and Svensson 2009, Rivera and

Andres 2002, Magurran and Seghers 1994). Another interesting empirical pattern is that tychoparthenogenesis — the rare development of unfertilized eggs in an otherwise sexually reproducing species — becomes common at low densities in Timema stick-insects (Schwander et al. 2010); without this capacity, the evolution and maintenance of parthenogenesis at low densities would obviously not be possible.

Our model has limitations: resisting females were assumed to all have the same strength of resistance θ. We therefore allow for trait evolution in the sense that the ratio from resisting to non-resisting females evolves, but do not include the possibility of continuous trait variation of θ. Letting θ evolve would require specifying how costs of resistance depend on the value of θ; further work could usefully consider the possibility of antagonistic coevolution between female and male behaviours, as higher θ might select for male counter adaptations.

We also did not consider any feedbacks between the proportion of parthenogenetic females in the population and the population density D. In reality, a higher proportion of parthenogenetic reproduction might lead to more efficient population growth. We have not included such feedbacks, because mate encounter rates in our model depend on adult density (at carrying capacity), which is a different population dynamic concept from the growth rate; to predict the latter purely based on the former is impossible without knowing how density regulation operates in any given species. That said, it is important to keep in mind that empirical testing of our evolutionary predictions regarding the relationship between parthenogenesis and population density could be complicated if males first disappear because density is low, and parthenogenetic females subsequently reach high densities. The dynamics of mating is especially important to understand when there is spatial variation (not yet included in our present model), which could take the form of an invasion front or then stochastic metapopulation dynamics. If it is possible that females over time reach higher densities when they can complete development without competing with male conspecifics, we can envisage an interesting 'double whammy' that makes maintaining males difficult as a whole: populations might then consist of regions where males are absent and regions where they are present. In the regions where males are present densities simultaneously are lowered (as less parthenogenesis means less population growth) – and low densities operate against male persistence, as we have shown. If the selective mosaic, however, also contains high-density patches where males are present, the outcome is less clear and might also depend on whether males or females disperse more.

Another interesting result of our model is that facultative sex is maintained at a wider range of densities if mate encounter rates increase less than linearly with mate availability (low β); at very high β the zone for facultative sex disappears. It would be intriguing to combine these findings with spatial dynamics, because of the point made in [30] that spatial clustering may lead to a smaller than linear increase of mating rates.

Finally, we did not consider any sex ratio adjustment (see (Kawatsu 2013*b*)) or a partial fertilisation of the eggs in the model. We assumed that after a female mated, all subsequent eggs are fertilized and offspring are produced at a 1:1 sex ratio. It has been shown that sex ratio evolution in female biased populations can increase the reproductive success of males and thus compensate for the cost of producing them and favour obligate sex (Kawatsu 2013*b*). On the other hand, the possibility of sex ratio adjustment could also lead to a lower fertilisation rate of the eggs for resistant females and even facilitate the extinction of males at lower densities.

Our findings also suggest that an interaction between male harassment and population density might contribute to our understanding of geographic parthenogenesis, an often documented pattern where asexual lineages appear to occupy marginal niches, with potentially low densities, compared with their sexual counterparts. One explanation for the pattern of geographical parthenogenesis is that asexual lineages are superior colonizers because they do not suffer from mate limitation (Baker and Baker 1965); see (Hörandl 2009) for this and alternative hypotheses. This is an interesting idea: it, too, features positive feedback, with the rarity of fertilization opportunities selecting for improved ability to reproduce without males, which further penalizes females who cannot do so (see (Schwander et al. 2010) for a discussion in the context of tychoparthenogenesis, i.e. the spontaneous development and hatching of unfertilized eggs in otherwise sexual organisms).

While these ideas and our model are not mutually exclusive, our model suggests that sexual harassment and female resistance at different population densities can lead to, or help maintain, geographic parthenogenesis, either independently or acting together with differences in colonization ability. In this scenario, a sexual species with some ability for parthenogenesis would first disperse to higher latitudes or altitudes. Thereafter different population densities in these habitats lead to the extinction or maintenance of males. If asexual types accompany the colonization process, the process will operate faster, if we assume that co-occurrence of asexual and sexual females reduces the local density of males at each point along the colonization route. Future work could therefore usefully investigate how mate limitation and sexual conflict operate together at niche margins *versus* elsewhere within a species' range.

Acknowledgements

We are grateful to Tanja Schwander and her group for stimulating discussions. Here we also want to thank three anonymous reviewers for helpful comments.

Data accessibility

The code for this project is available on GitHub: https://github.com/nigerb/TimemaCode

Authors' contributions

NG and HK conceived the study; NG and HK constructed the model and analysed the data NG HK wrote and edited the manuscript; all authors revised the manuscript and gave final approval for publication.

Appendix Chapter VI

Parameter	Description	Explored range
θ	Strength of resistance	$[\mathbf{1}, \mathbf{50}]$
α	Relative cost of resistance of R females	$[\mathbf{0}, \mathbf{1}]$
β	Exponent of male encounter rate	$[\frac{\mathbf{1}}{\mathbf{2}}, \mathbf{2}]$
p0	Initial frequency of R in the population	[0, 1]
f0	Initial frequency of females	[0, 1]

TableS1 model parameters

Influence of θ and α

The densities at which females are selected to 'accept' a sexual life-cycle upon encountering a male, and from which density upwards males can exist, depend on the strength of resistance θ and the costs of resistance α (Figures S1, S2) in addition to the parameters discussed in the main text.

At very low density, neither θ nor α influence the outcome: only females persist, with the initial frequency determining which type of female exists at the evolutionary endpoint (the neutral case). A similar independence of θ and α exists at the high end of densities: *de facto* sexuality arises here regardless of the values of these parameters (Figures S1, S2).

Intermediate densities reveal more complex patterns. With increasing costs of resistance α, the density threshold at which it pays for females to 'mate for convenience' is shifted to lower population densities (Figure S2). This applies for both low and high initial frequencies of *R.* The effect of θ at intermediate densities is more complex. If the initial frequency of *R* is high, the density threshold required for 'mating for convenience' increases with increasing θ (Figure S1), but if it is low, then the density threshold is instead lowered with increasing θ (Figure S1). In other words, the point from which *r* individuals can take over a *R* population can become shifted toward either higher or lower densities when θ increases (Figure S1).

Figure S1 The influence of the strength of resistance, θ, on the population composition at equilibrium. Colours indicate the occurrence of *r* and *R* males and females for a combination of values of population density, *D*, and the efficiency of resistance, θ. The colour scheme as a whole is arranged such that blue shades indicates the occurrence of males independent of the initial genotype frequencies, whereas in the purple area males existence depends on the initial genotype frequency. Each outcome is shown combining information from 2 runs: an initial frequency of *R* individuals of 0.001 and another of 0.999. If the legend associates a given colour with just one outcome, then this outcome is reached from both initial frequencies. Several of the colours yield two outcomes depending on initial frequencies: for example, at population density D = 300 and θ = 20, males and females persist at equilibrium, but the identity of the persisting genotype depends on the initial genotype frequency. Note that θ does not impact the outcomes if density is very low or very high. Parameters used: $\alpha = 0.01$ and β = 1.

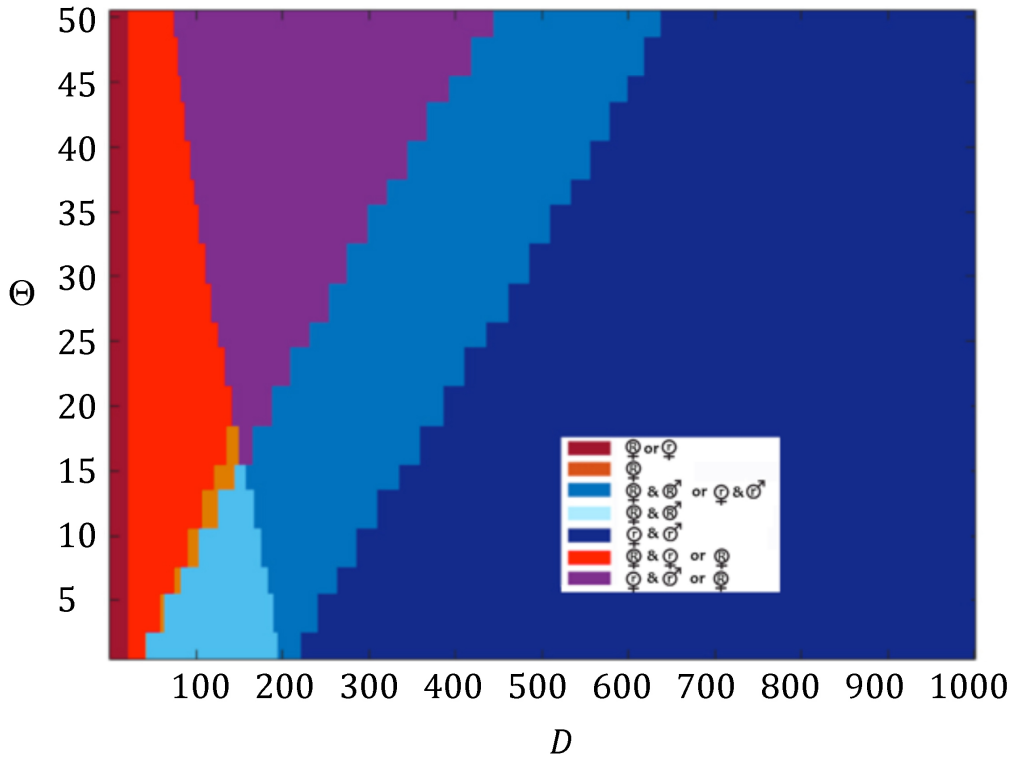


Figure S2 The influence of the costs of resistance $\boldsymbol{\alpha}$ on the population composition at evolutionary equilibrium, for different values of population density D. The outcome is shown combining information from an initial frequency of R individuals of 0.001 and 0.999, with the colour indicating the possible outcomes, similarly to Figure S1. For example, at population density D = 200 and $\boldsymbol{\alpha}$= 0.02, males and females persist at equilibrium, but the persisting genotypes depend on the initial genotype frequency. Blue colour indicates the occurrence of males independent of the initial genotype frequencies. At very low and high densities there is no influence of $\boldsymbol{\alpha}$. Other parameters: $\theta = \mathbf{5}$ and β = 1.

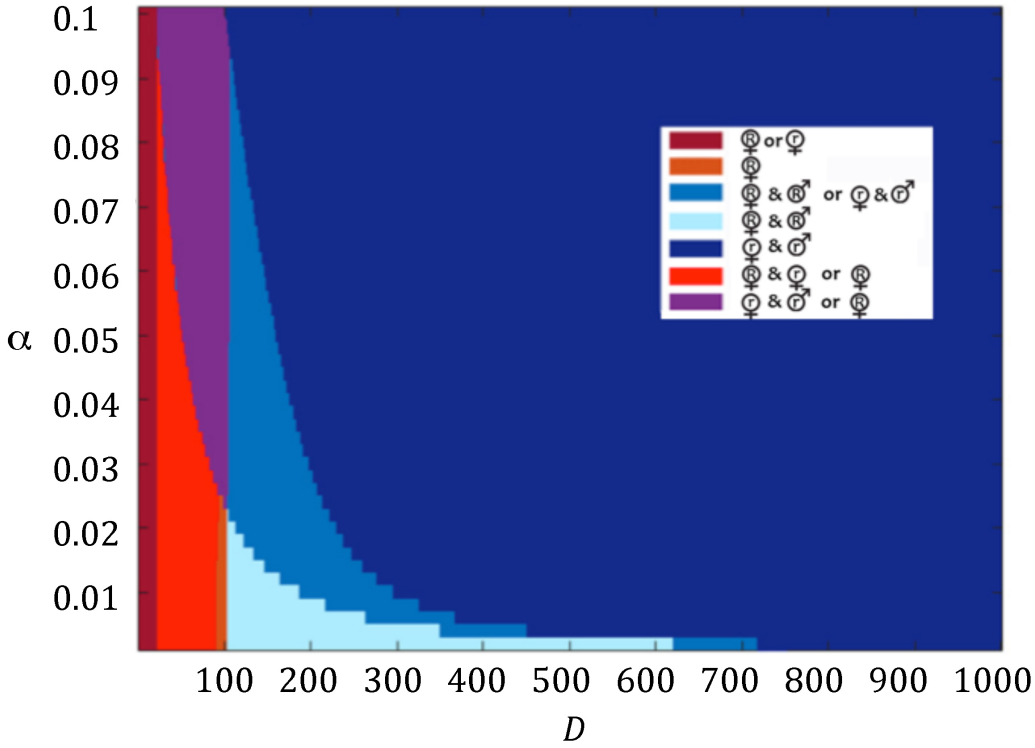


Figure S3 The influence of the initial frequency of the *R* allele on the composition of the population at evolutionary equilibrium as indicated by the colour. Where the colour changes when following a vertical line (e.g. at D = 20), the outcome is dependent on the initial frequency, whereas uniform colour along any vertical line (e.g. D = 50) indicates a single outcome regardless of the initial frequency. Other parameters: $\theta = 5$, α=0.01, β = 1.

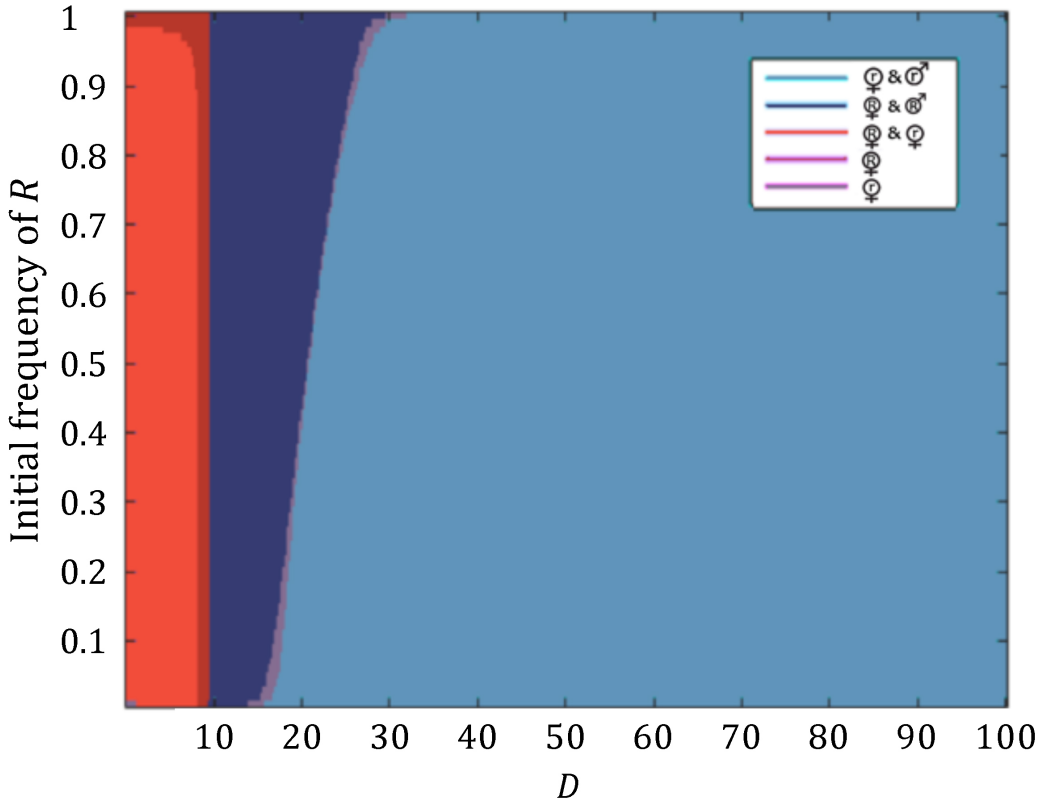


Figure S4. The evolved sex ratio at the end of 1000 generations based on simulating 10000 different uniformly distributed random parameter values, where θ was varied between 1 and 10, α between 0 and 0.01, the initial frequency of R between 0.001 and 0.999, and population density (plotted on the x axis) between 1 and 100. We used the null model assumption $\beta = 1$. Darker coloration indicates overlapping points (the darker the colour, the more points coincide at this location, up to 98 cases for the darkest colour present in the figure).

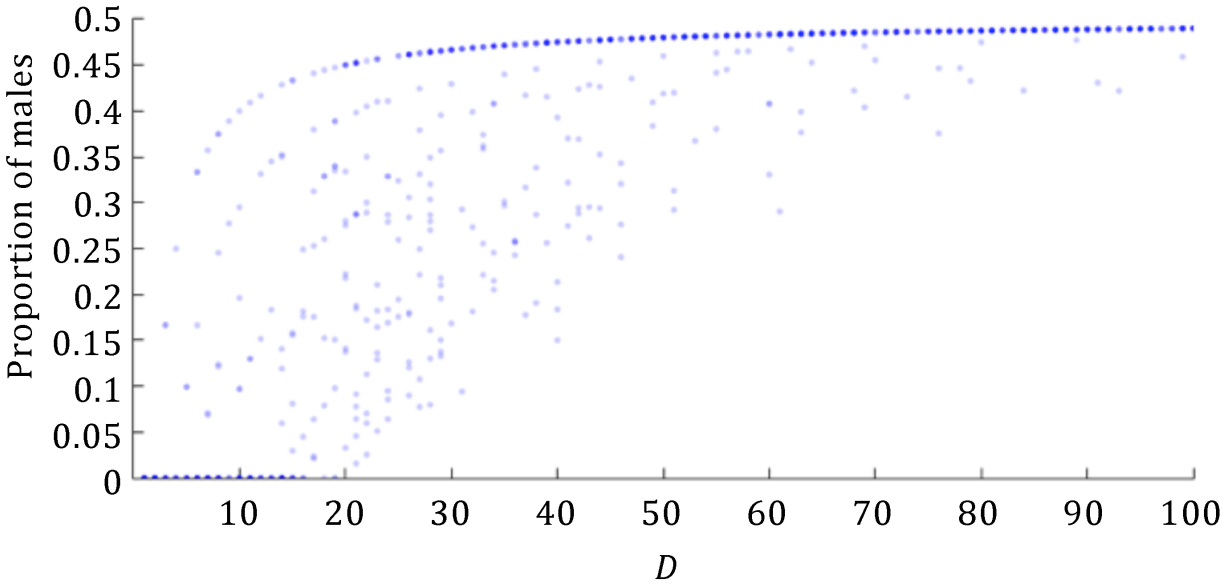


Figure S5 The influence of β (the scaling between density and mate encounter rate) on the population composition, for five values of β from $\frac{1}{2}$ to 2 as indicated. When β is low male encounter rate and population density are related in a non-linear way and both, low and high population densities resemble intermediate densities in terms of mate encounter rate. Thus prolonged mate encounters (low β) facilitate the evolution of sex in this system. Note that population density D ranges up to 10^3 and is illustrated on a logscale. Other parameters: $\theta = 5$, $\alpha = 0.01$.

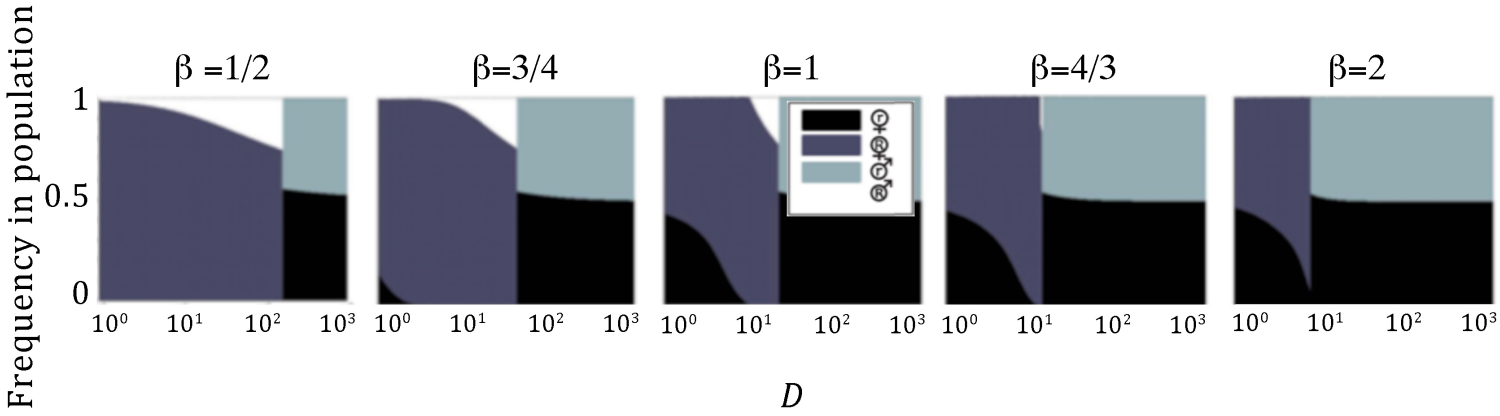


Figure S6 A comparison of the effects of initial sex ratio on the population composition as a function of population density at stable evolutionary endpoints, for an initial frequency of *R* individuals of 0.001 (a, d), equal initial frequency for both types (b, e) and for an initial frequency of *R* individuals of 0.999 (c, f). The subplots on the left (a-c) show the results for an initial high male frequency (0.999) whereas the subplots on the right (d-f) are generated with an initially low frequency of males (0.001). Other parameters: $\theta = 5$, $\alpha = 0.01$, $\beta = 1$.

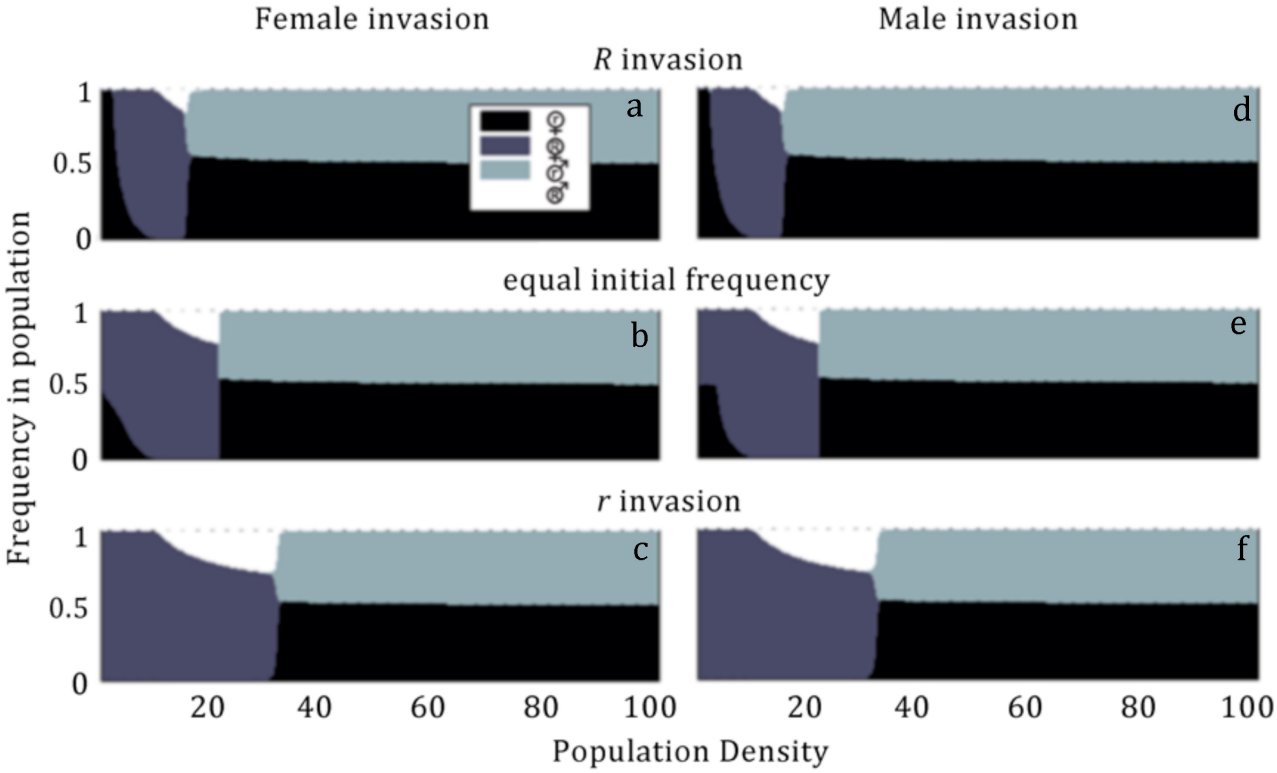


Figure S7 The population composition at evolutionary endpoints when α = 0, which equalizes the mortalities for the two female types, (a) for an initial frequency of *R* individuals of 0.001, and (b) for an initial frequency of *R* individuals of 0.999. This figure shows what happens, when cost of resistance disappear. As soon as males can persist in the population, resistance pays off and becomes fixed. The non-resistant type can only persist at densities where males go extinct. Other parameters: $\theta = 5$, $\alpha = 0$, β = 1.

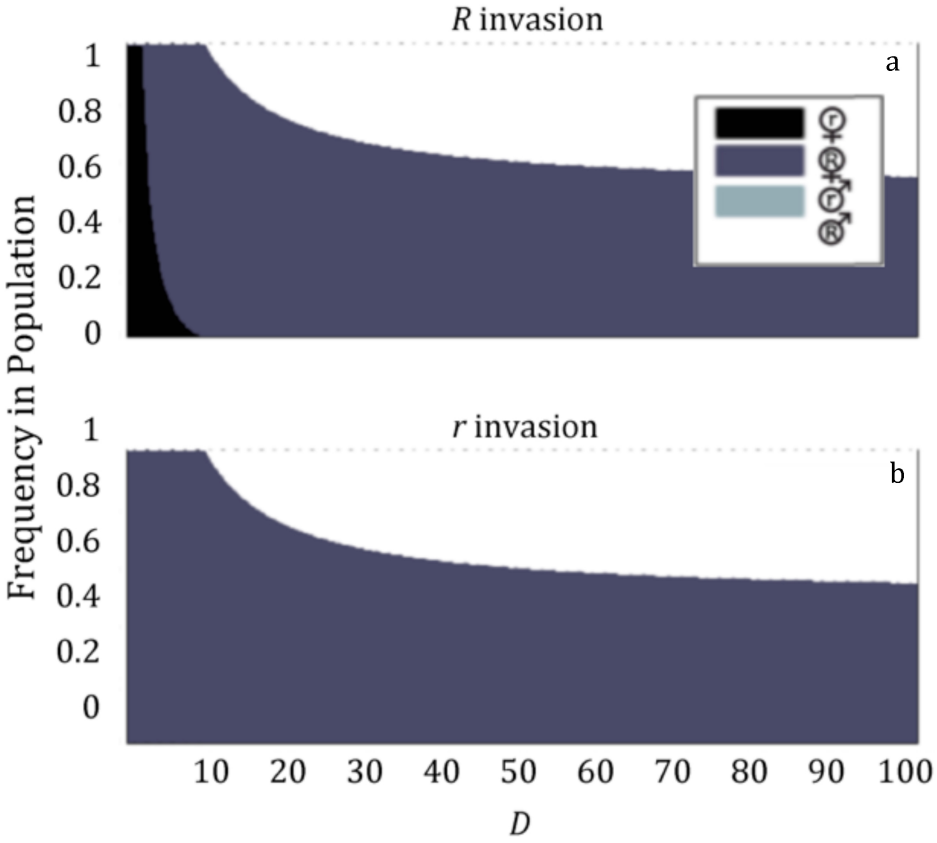
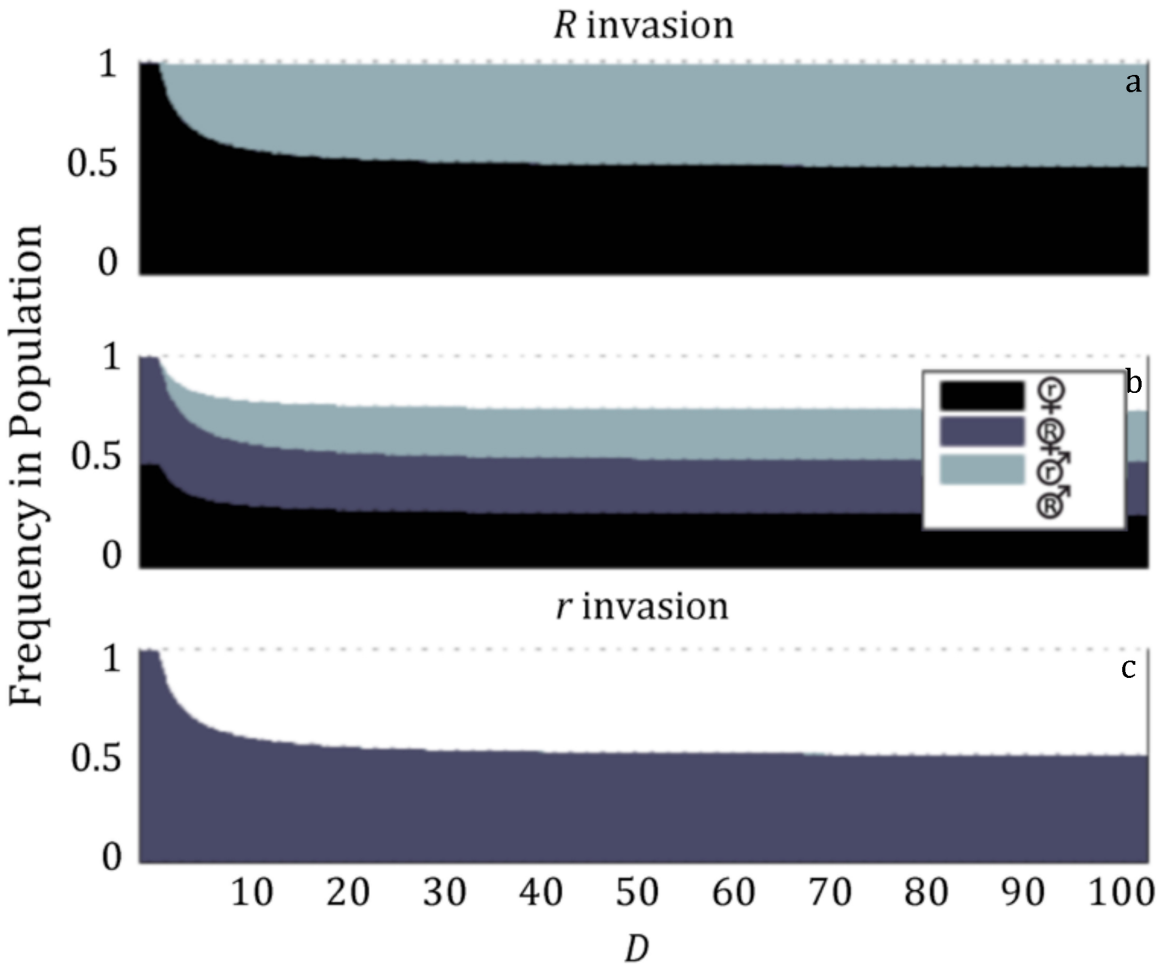


Figure S8 The evolutionarily stable composition of the population for the special case α = 0 and $\theta = 1$. Frequencies of *r* and *R* males and females as a function of population density at stable evolutionary endpoints, (a) for an initial frequency of *R* individuals of 0.001 and (b) for an initial frequency of *R* individuals of 0.999. Here, matings do not involve any sexual harassment, and the genotypes only differ in the initial frequency. Without female resistance males go extinct at very low population density due to 'self-extinguishing' male demography. Other parameters: $\theta = 1$, $\alpha = 0$, β = 1.



CHAPTER VII

General discussion

This thesis aimed to investigate the causes and consequences of facultative sex and to connect these findings to the maintenance of sexual reproduction. I investigated the consequences of facultative sex regarding sex allocation and the timing of sex by modeling optimal behavior (Chapter II), following population dynamics of natural *Daphnia magna* populations (Chapter III), and performing mesocosm experiments (Chapter IV). Furthermore, I investigated the co-evolution of sexual reproduction with dispersal and dormancy using individual-based simulations and by integrating condition-dependent investment in escape traits in a bet-hedging framework (Chapter V). Lastly, using demographic calculations, I investigated the effect of sexual conflict on the maintenance of sex in dependence of population density (Chapter VI).

Sex allocation and the timing of facultative sex (Chapters II-IV)

In facultative sexual organisms, which can alternate between sexual and asexual reproduction, there is strong selection on the timing of sexual reproduction within a growing season: If you can do both, when should you invest in which mode of reproduction? In the *Daphnia* system, the major cost of sexual reproduction stems from demographic consequences of sex: Females that sexually produce an ephippium with two eggs cannot produce an asexual clutch – which can contain up to 110 eggs – at the same time, leading to reduced competitiveness within the season. A similar cost to asexual growth comes from producing a clutch of male, rather than female, offspring. To maximize fitness, the timing of sexual reproduction is thus expected to optimize investment in the two reproductive modes.

Not only demographic consequences shape the timing of sex, but also seasonality of the environment and the predictability of inhospitable periods. In organisms where sex is linked with the production of a resting stage, such environmental factors influence the timing of sex together with demographic pressures. Here, I showed that for facultative sexual organisms, temporally spreading the induction of sexual reproduction – and with this the production of dormant eggs – is selected for in unpredictable environments. However, in predictable seasons, sexual production of dormant eggs is much more precise, and occurs only shortly before the season ends.

These results are consistent with earlier models on cyclical parthenogens: A general model of a cyclical parthenogen life-cycle demonstrates a gradual induction of sex in uncertain environments (Spencer et al. 2001). Also, in other cyclical parthenogens, such as aphids, optimal induction of sexual reproduction is proportional to variance in season length (e.g. Halkett et al. 2004). Our model predictions also match empirical patterns from natural populations. In predictable environments (e.g. temperate lake populations), *Daphnia* produce ephippia only towards the end of the breeding season (Galimov et al. 2011), whereas *Daphnia* in ephemeral habitats (e.g. shallow ponds in northern latitudes) produce ephippia throughout the growing season (Altermatt and Ebert 2008, Roulin et al. 2013, Chapter III).

When we add density-dependent population growth to this picture, populations are predicted to induce sexual reproduction as soon as population density peaks, in addition to adjusting the investment in sex to the approaching season end (Chapter II). This pattern is well supported by our empirical results: In our sampled populations, sexual reproduction was strongly predicted by population density. A greater proportion of females engaged in sexual reproduction when density was high at the previous sampling point (Chapter III).

This association of sex with high population densities is not *Daphnia* specific. It has been documented in other facultative sexuals, e.g. Rotifers (Gilbert 1963, Steltzer & Snell 2003, Serra et al. 2008). An association of sex with high population density generally fits the habitat deterioration hypothesis: Sex should be induced shortly before periods of high mortality (e.g. Serra & Carmona 1993), as high densities might anticipate habitat deterioration. However, here, we suggest that an increase of sexual reproduction at high densities results from reduced relative cost of sex when population densities are high rather than from approaching inhospitable conditions (Chapter III). In the populations we observed, periods of high densities were not followed by inhospitable conditions and populations recovered after a crash. However, at high population densities, the efficiency of asexual reproduction was decreased – juveniles might have difficulty recruiting into the population when density (and with this competition) is already high. Sexually produced offspring do not experience these difficulties, as they only hatch in the next season and thus are not affected by the current conditions. Thus, the opportunity cost of sex – due to missing out on asexual reproduction – is reduced at high population densities, because asexual reproduction is inefficient (Chapter III).

Regarding sex allocation, our model predicts that male production should always be induced before sexual reproduction, as mature males are needed for sexual reproduction. However, male production is also expected to co-occur with sexual reproduction when information on the timing of the season end is imperfect. Furthermore, our results highlight that sex ratios close to 1:1 only occur exceptionally in situations where all females will switch to sexual reproduction in the near future (Chapter IV). These results fit with sex allocation theory, where overlapping generations and sequential decisions with mortality occurring in-between lead to predictions deviating from equal investment (West 2009, Kahn et al. 2015). Additionally, facultative sex leads to predictions towards fewer males than expected for systems with equal investment.

But not only the timing of sex (and thus the need for males) might shape sex allocation: Our results show that the timing of male production is not only influenced by drivers of the timing of sex (e.g. population density), but also by the relative pay-off of males depending on the current sex ratio of the population. In our mesocosm experiment, females produced more sons at higher densities, but this effect was dampened in male biased populations (Chapter IV).

For the timing of sex in facultative sexual organisms, where sex is linked with a resistant stage, we can conclude that populations that live in seasonal habitats should invest more in sex than populations in permanent habitats (Roulin et al. 2013). Within a season, the investment in sex should be proportional to the variance in the occurrence of the end of a season. In unpredictable environments, investment in sexual reproduction should spread over several breeding cycles within the season for individuals to hedge their bets. However, in predictable environments, sex should occur just before the season ends. Furthermore, the

within season pattern is not only shaped by the predictability of the onset of the unfavorable season, but also shaped by a trade-off between the reproductive modes: Sex should be performed when its costs are relatively low compared to asexual reproduction. This is the case at high population densities, when asexual reproduction becomes less efficient. Thus, the timing of sex and sex allocation in cyclical parthenogens is not only shaped by the timing of inhospitable conditions, but also shaped by the relative cost of sexual vs. asexual reproduction and the relative profitability of males vs. females. When seeking explanations for the maintenance of sex, predictions of when sex occurs should consider the relative cost of sex as well as variation through e.g. population dynamic consequences.

Facultative sex and the evolutionary stability of sex (Chapters V-VI)

Why sex is so prevalent is one of the main questions in evolutionary biology (Bell 1982). Theory predicts that in the short term sex should be outcompeted by asexual reproduction because of the many costs of sex (Maynard Smith 1978, Lehtonen et al. 2012, Otto 2003). One mechanism that can prevent a sexual population's invasion by a purely asexual strategy, and can thus stabilize sex (or facultative sex), is when sex is linked to an ecological function such as dispersal or dormancy (Stelzer & Lehtonen 2016). Links between sexual reproduction and the production of a dormant or dispersing life stage are common across many facultative sexual species (e.g. *Daphnia* (Ebert 2005), rotifers (Carmona et al. 2009), aphids (Simon et al. 2002)). However, there is no *a priori* explanation for this association. Here, I investigated why such a link should evolve in the first place. Sex, dispersal and dormancy all reflect bet-hedging strategies, and theory predicts that investment in one bet-hedging trait should reduce investment in any other bet-hedging trait (Starrfelt & Kokko 2012). By incorporating condition dependence in a classical bet-hedging framework, we could show that within a species, a positive correlation of these traits can arise (Chapter V). Thus, we provide an explanation for the evolution of a link of sex with dispersal or dormancy, which can stabilize sex and prevent the invasion of asexual lineages into a sexual population.

Another mechanism that can stabilize sex is sexual conflict. At first sight, sexual conflict and sexually antagonistic co-evolution should add to the cost of sex because of negative effects that males might have on females (Rankin and Kokko 2007). However, in facultative sexual species, where females can reproduce asexually and sexually, females trying to reproduce sexually do not necessarily escape harmful male behaviour (Dagg 2006). Especially at high population densities, where male encounter rates are high, male harassment can lead to high costs for females that try to resist male mating attempts. In this thesis, I show that sexual conflict can stabilize sex at high population densities, because at high densities resistance does not pay off for females (Chapter VI). At low population densities, on the other hand, asexual reproduction and the extinction of males evolve. At low densities, male encounter rates are low and thus the costs of resistance are outweighed by the benefits of asexual reproduction (Arnqvist & Rowe 2013). Facultative sex only persists at a narrow, intermediate range of population density (Chapter VI). Thus, at high population densities, the ability to reproduce asexually can be lost. Under these conditions, obligate sex evolved from facultative sex as a consequence of sexual conflict.

In this thesis, I investigated the conditions under which sexual reproduction can persist or spread in a population, in competition with asexual reproduction. When evaluating the

maintenance of sex it is important to consider the cost and benefits of sex in a system, where individuals can use both reproductive modes. This gives a different perspective to the problem compared to when only the obligate forms are considered. I could provide insights into the evolution of a link between sex and dispersal or dormancy – a mechanism that prevents asexual invasion and thus allows the maintenance of sexual and facultative sexual reproduction in the face of short term benefits of asexual reproduction. I could further show that an obligate sexual strategy can invade a facultative sexual strategy as a consequence of the interaction of sexual conflict and high population densities. Thus, I could shed light on mechanisms that stabilize obligate sexual reproduction against the invasion of asexual as well as facultative sexual lineages.

What can we learn from the *Daphnia* system?

The *Daphnia* system at the southern coast of Finland proved to be an ideal system to investigate the causes and consequences of facultative sex. First, the system offers interesting dynamics that shape the evolution of the timing of sex and sex allocation: In *Daphnia magna,* sex is linked with the production of dormant eggs, which influences the trade-offs important for the timing of sexual reproduction and allows the integration of sex allocation with bet-hedging theory.

Second, *Daphnia* present a great experimental system to investigate the consequences of facultative sex and test predictions of sex allocation theory in cyclical parthenogens. With overlapping generations and temporal variation in the sex ratio, *Daphnia* fulfil the theoretical assumptions for facultative adjustment of sex allocation depending on the current sex ratio in the population (see chapter IV). Furthermore, the short generation time and the potential for very fast growth allow for high sample sizes and the completion of experiments in manageable time periods. Males and females can be distinguished rather easily, allowing for easy manipulation of sex ratios. Additionally, in laboratory experiments, clonal sisters can be used to control for genotype differences, and the production of sexual eggs can be easily be triggered through e.g. photoperiod differences (See chapter III).

There are a few studies on sex allocation in other cyclical parthenogens, but these studies consider either sex allocation or bet-hedging, but not both in combination. In particular, sex allocation in rotifers has been studied quite well (Aparici et. al. 1998 & 2002, Serra et al. 2008). However, in contrast to haplodiploid rotifers, *Daphnia* females do not commit irreversibly to sexual reproduction. Instead, they can switch back and forth between asexually producing females, asexually producing males, and sexually producing an ephippium. These life history differences result in different dynamics of sex allocation in *Daphnia* compared to the rotifer system: sex allocation in rotifers is directly related to male frequency, whereas in *Daphnia,* we found indirect effects of the relative frequency of males on the production of sons and daughters. Still, despite differences in the details, broad similarities in patterns of sex allocation can be seen across not only these different facultative sexual systems, but also obligate sexual systems in which sex allocation theory was originally developed. My thesis illustrate how incorporating the specific demographic parameters of particular organisms into tests of theory can allow identification of general processes, as well as of the system-specific details that may explain divergence from general predictions.

As mentioned above, previous studies of sex allocation in systems similar to *Daphnia* do not consider variation in season length or bet-hedging. Other studies that consider the timing of

facultative sex in a bet-hedging framework (e.g. Halkett et al. 2004, Tarazona et al. 2017) do not consider sex allocation. In this thesis, I linked these two fields and integrated the bet-hedging framework with sex allocation in a *Daphnia*-like system by modelling the optimal strategy of when to produce males and mate with them. This combination allows investigating the dynamics, when not only sexual reproduction but also male production can evolve in response to unpredictable environments. This leads to two different patterns: sex and male production can either co-occur or being temporally separated within a season, depending on whether direct or indirect cues for the season end are available

In *Daphnia*, population density seems to be associated with conditions favouring sexual reproduction. Again, this is not a *Daphnia* specific pattern (e.g. rotifers (Stelzer and Snell 2003, Serra et al. 2008). High population densities might be a cue for the upcoming season end, and thus represent a cue for the induction of production of resting stages. High population densities are also associated with high competition and low resource availability, and thus with stressful conditions, which are known induce sexual reproduction (see Ram & Hadany 2016 for a recent review). Furthermore, at high population densities, mate encounter rate is increased – a condition favouring sex (Snell and Garman 1986). Here, I found that at high population densities, the relative costs of sexual reproduction are reduced, due to lower efficiency of asexual reproduction. This leads to an association of male production and sexual reproduction at high population densities. High population density seems not only to be associated with sexual reproduction in the seasonal dynamics of facultative sexual organisms, but also in general represents conditions where sex is favored. When considering sexual conflict, I found that high population densities favoured the evolution of obligate sex, while obligate asexuality was associated with low population densities and facultative sex with a small range of intermediate population densities (Chapter VI). In both the timing of sex and sexual conflict scenarios, population density was an important determinant of the pay-off of different reproductive modes.

By investigating the *Daphnia* system, we can draw general conclusions for the evolution of the timing of sex: (i) In a system where sex is linked to dormancy (which is the case in many facultative sexual species), females evolved to adjust the induction of male production and sex to the variation in the season length, and hedge their bets in unpredictable environments. Furthermore, in density-dependent populations, sex was induced when densities are high, independent of the probability for the approaching season end (Chapter II). Thus demographic processes feed back to the evolution of the optimal timing of sex. (ii) Investment in sex increased when its relative costs were reduced, which coincided with high population densities (Chapter III). (iii) Predictions for conditional sex allocation hold true in a system where sex is facultative, and that *Daphnia* females do not only adjust their sex ratio to population density, but adjust it further in relation to the current sex ratio in the population (Chapter IV). (iv) In a *Daphnia*-like system, different co-evolution patterns of sex, dispersal and dormancy can arise when considering within vs. across species patterns (Chapter V).

What can we learn from a facultative sexual system?

Why most eukaryotes engage in sexual (vs. asexual) reproduction is still an important question in evolutionary biology. Systems in which both sexual and asexual reproduction are performed can provide a powerful way of addressing the question of the stability of sex. In facultative sexual populations, it is possible to observe the balance of sexual and asexual repro-

duction and determine conditions for when sex is favoured. Important insights into the cost and benefits of sex can be gained by characterizing when and under what conditions these facultative sexual organisms engage in sexual vs. asexual reproduction.

In this thesis, investigating the conditions under which *Daphnia* switch to sexual reproduction, I could show that the cost of sex is fundamental to the predicted occurrence of sex. Investment in sex increased when its relative costs were reduced due to the underlying population dynamics. I suggest that, when seeking for explanations of the maintenance of sex, one should consider the relative cost of sex and variation in this cost.

Furthermore, when trying to explain the maintenance of sex, sexual and asexual reproduction should not be treated as mutually exclusive (Burke & Bonduransky 2017). Facultative sexual strategies are often referred to as strategies combining "the best of both worlds" and should outcompete obligate sexual or asexual strategies, because occasional sex is sufficient to provide the benefits of sex, while most of the costs can be avoided (Green & Noakes 1995, D'Souza and Michiels 2010, Hurst & Peck 1996). Furthermore, in many facultative sexual systems, sex is performed when needed and often depending on current conditions (Ram & Hadany 2016). Strategies using occasional sex should therefore outperform obligate strategies. This means that explaining conditions under which sex can persist against obligate asexuality does not explain the maintenance of obligate sex, because a facultative strategy could still be superior. However, while facultative sex seems to be widespread in unicellular eukaryotes (Dacks & Roger 1999), most multicellular eukaryotes are obligate sexuals (Bell 1982*).* Thus, as stated by (Burke & Bonduransky 2017), the real paradox of sex should not be the prevalence of sex, but why facultative strategies are so rare in multicellular eukaryotes, respectively, why did obligate sex evolve at all, given that theoretically, facultative sex seems to be superior to the obligate strategy? To understand this, we need to investigate the factors that prevent facultative sexual organisms from invading populations with obligate sex.

In this thesis, I have suggested one potential explanation: as long as males exist, it is in their interest that sexual females are maintained in a population. If males are able to influence female reproductive mode and 'enforce' sexual reproduction, it may be too costly for females to resist. Thus, in a system where females can reproduce sexually or asexually, sexual conflict can stabilize obligate sex. It is usually assumed that asexual lineages can escape the costs of males, both in terms of producing sons and of costs such as mate searching and sexual harassment by males under sexual selection. However, this is not necessarily true – if asexual lineages share their environment with sexual relatives, and males harass females irrespective of their type, asexual females will also suffer from the costs of male harassment. In such a scenario, I showed that facultative sex is only stable over a narrow range of intermediate population densities, whereas obligate sexual reproduction outcompetes facultative strategies at high population densities. Thus, I could identify conditions where obligate sex is superior to a facultative strategy (Chapter VI).

In general, the timing and frequency of sexual reproduction are critical traits determining the trade-off between sexual and asexual reproduction, and the optimal investment in each reproductive mode. This optimal investment can be influenced by environmental as well as demographic factors, as we show for the *Daphnia* case (Chapter II-III). However, this trade-off also includes the risk that facultative sexual reproduction might be unstable in the long term (D'Souza and Michiels 2010), leading to obligate asexuality or sexuality. Other explanations for why many eukaryotes evolved obligate sex include genetic or developmental con-

straints that prevent the evolution of facultative sex from the obligate sexual reproduction hypothesised in the last common ancestor of the eukaryotes. Especially genetic imprinting is often referenced as the reason why any form of asexual reproduction is absent in mammals (Solter 1988, Engelstädter 2008).

There are also mechanisms that can prevent the evolution of obligate asexuality and thus stabilize sex (in its occasional form), for example the commonly observed link between sexual reproduction and an ecological function such as dormancy or dispersal (Williams 1975, Castel et al. 2014). Here I modelled the evolution of such a link and I could show that the patterns of co-evolution of sex, dispersal and dormancy can differ, depending on whether within- or across-species patterns are considered. By combining classical bet-hedging theory with condition-dependency, I provided an explanation for the discrepancy between theoretical predictions and empirically observed associations between multiple bet-hedging traits (Chapter V).

In sum, a consideration of facultative sexual reproduction aids in understanding the maintenance of obligate sex. My thesis, focusing on facultative sexual systems, allowed me to (i) investigate conditions under which sex is favoured, and (ii) explain why obligate sexual populations are not invaded by facultative sexual strategies. Additionally, understanding facultative sex is interesting in its own right, especially given the predominance of this reproductive system across the eukaryotes.

General conclusions

In this thesis I could show that the relative cost of sex should be considered when seeking explanations for the maintenance of sex and testing the predictions of long standing theory. I identified conditions for the co-evolution of sex with dispersal or dormancy – a commonly observed association, which stabilizes sex in facultative sexual systems, but which is not predicted by classical bet-hedging theory. Furthermore, in a model considering sexual harassment, I could provide potential explanations for the prevalence of obligate sex. My results suggest that future work should take into account sex specific patterns and sexual selection when seeking explanations for the widespread of obligate sex – the true paradox of sex (Burke & Bonduransky 2017).

This thesis investigated the causes and consequences of a strategy that combines different reproductive modes by combining empirical and theoretical methods and integrating different fields – bet-hedging with sex allocation and condition-dependency. This was not just an interesting mix of methods and ideas, but also proved to be rather fruitful. By integrating different theoretical frameworks, and combining theory with empirical tests, I could shed light on discrepancies between previous theoretical predictions and common empirical observations. I connected bet-hedging with sex allocation theory to investigate the consequences of facultative sex, and by incorporating condition-dependency in classical bet-hedging theory, I could identify conditions where a positive correlation of sex, dispersal and dormancy evolves. I think that the combination of theoretical and empirical work along with synergisms of classical theories will help to answer still open questions and help us to understand why such a variety of different reproductive systems exists.

Bibliography

Aanen, D. K., and R. F. Hoekstra. 2007. Why Sex Is Good: On Fungi and Beyond. Pages 527–534 in J. Heitman, J. W. Kronstad, J. W. Taylor, and L. A. Casselton, eds. Sex in fungi Molecular Determination and Evolutionary Implications. American Society of Microbiology.

Aanen, D., M. Beekman, and H. Kokko. 2016. Weird sex: the underappreciated diversity of sexual reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20160262–4.

Abrahamson, G. W. 1980. Demography and Vegetative Reproduction. (O. T. Solbrig, ed.).

Alekseev, V., and W. Lampert. 2001. Maternal control of resting-egg production in Daphnia. Nature 414:899–901.

Allsop, D.J., Warner, D.A., Langkilde, T., Du, W., Shine, R. 2006. Do operational sex ratios influence sex allocation in viviparous lizards with temperature-dependent sex determination? *J. Evol. Biol.* 19, 1175-1182.

Altermatt F, Ebert D. 2008 The influence of pool volume and summer desiccation on the production of the resting and dispersal stage in a *Daphnia* metapopulation. *Oecologia* 157, 441-452. (doi:10.1007/s00442-008-1080-4)

Aparici E, Carmona MJ, Serra M. 1996 Polymorphism in bisexual reproductive patterns of cyclical parthenogens: a simulation approach using a rotifer growth model. *Ecol. Model.* 88, 133-142.

Aparici, E., Carmona, M.J., Serra, M. 2002. Evidence for an even sex allocation in haplodiploid cyclical parthenogens. *J. Evol. Biol*. 15, 65-73.

Aparici, E., M. J. Carmona, and M. Serra. 1998. Sex Allocation in Haplodiploid Cyclical Parthenogens with Density-Dependent Proportion of Males. The American Naturalist 152:652–657.

Arnqvist, G., and L. Rowe. 2013. Sexual Conflict. Princeton University Press.

Asker, S. E., and L. Jerling. 1992. Apomixis in plants. CRC, Boca Raton, FL.

Auld, S.K.J.R., Tinkler, S.K., Tinsley, M.C. 2016. Sex as a strategy against rapidly evolving parasites. *Proc. R. Soc. B*. 283, 20162226.

Baker, H. G., and H. G. Baker. 1965. Characteristics and Modes of Origin of Weeds. Pages 147–186 inThe genetics of colonizing species.

Barker, D.M., Hebert, P.D.N. 1986. Secondary sex ratio of the cyclic parthenogen *Daphnia magna* (Crustacea: Cladocera) in the Canadian Arctic. *Can. J. Zool*. 64, 1137-1143.

Barnes, R. D. 1982. Invertebrate Zoology. Philadelphia, PA: Holt-Saunders International.

Bates D, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw*. 67, 1-48.

Begon M, Harper JL, Townsend CR. 1990. *Ecology (2nd Ed.)* Blackwell Scientific, Cambridge MA, pp 150-152.

Bell, G. 1982. The masterpiece of nature: The Evolution and Genetics of Sexuality. University of California Press

Bengtsson O. B., and A. Ceplitis. 2000. The balance between sexual and asexual reproduction in plants living in variable environments. Journal of Evolutionary Biology 13:415–422.

Bengtsson, O. B. 2009. Sex and evolution: A very large-scale overview. In I. Schon, K. Martens, & P. van Dijk (Eds.), Lost sex: The evolutionary biology of parthenogenesis (pp. 1–19). New York: Springer.

Bensch, S., Westerdahl, H., Hansson, B., Hasselquist, D. 1999. Do females adjust the sex of their offspring in relation to the breeding sex ratio? *J. Evol. Biol*. 12, 1104-1109.

Berg, L.M., Palsson, S., and Lascoux, M. 2001. Fitness and sexual response to population density in Daphnia pulex. Freshwater Biol. 46, 667-677.

Beukeboom, 2014 L. W., N. Perrin,. The Evolution of Sex Determination. Oxford University Press.

Bierzychudek P. 1985 Patterns in plant parthenogenesis. Experientia 41,1255–1264. (doi:10.1007/BF01952068)

Billiard, S., M. Lopez-Villavicencio, M. E. Hood, and T. Giraud. 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. Journal of Evolutionary Biology 25:1020–1038.

Bond, M.L., Wolff, J.O., Krackow, S. 2003. Recruitment sex ratios in gray-tailed voles (*Microtus canicaudus*) in response to density, sex ratio, and season. *Can. J. Zool*. 81, 1306-1311.

Bonner, J. T. 1958. The Relation of Spore Formation to Recombination. The American Naturalist 92:193–200.

Bonte, D., H. Van Dyck, J. M. Bullock, A. Coulon, M. Delgado, M. Gibbs, V. Lehouck, et al. 2011. Costs of dispersal. Biological Reviews 87:290–312.

Booksmythe I, Gerber N, Ebert D, Kokko H. *under review. Daphnia* females adjust sex allocation in response to current sex ratio and density. Ecology Letters

Bradford, M. J., and D. A. Roff. 1993. Bet-hedging and the Diapause Strategies of the Cricket Allonemobius Fasciatus. Ecology 74:1129–1135.

Brewer, M.C. 1998. Mating behaviours of *Daphnia pulicaria*, a cyclic parthenogen: comparisons with copepods. *Phil. Trans. R. Soc. Lond. B*. 353, 805-815.

Brown, L.P. 1982. Can guppies adjust the sex ratio? *Am. Nat*. 120, 694-698.

Buoro, M., and S. M. Carlson. 2014. Life-history syndromes: Integrating dispersal through space and time. (J. Clobert, ed.)Ecology Letters 17:756–767.

Burke, N. W., A. J. Crean and R. Bonduriansky. 2015. The role of sexual conflict in the evolution of facultative parthenogenesis: a study on the spiny leaf stick insect. Animal Behaviour 101:117–127.

Burke, N. W., and R. Bonduriansky. 2017. Sexual Conflict, Facultative Asexuality, and the True Paradox of Sex. Trends in Ecology & Evolution 32:646–652.

Burt A. 2000 Perspective: Sex, recombination and the efficacy of selection – was Weismann right? *Evolution* 54, 337-351. (doi:10.1111/j.0014-3820.2000.tb00038.x)

Byholm, P., Ranta, E., Kaitala, V., Linden, H., Saurola, P., Wikman, M. (2002). Resource availability and goshawk offspring sex ratio variation: a large-scale ecological phenomenon. *J. Anim. Ecol.* 71, 994-1001.

Caprioli M., C. Ricci. 2001. Recipes for successful anhydrobiosis in bdelloid rotifers. Hydrobiologia 446: 13. https://doi.org/10.1023/A:1017556602272

Carmona, M. J., N. Dimas-Flores, E. M. Garcia-Roger, and M. Serra. 2009. Selection of low investment in sex in a cyclically parthenogenetic rotifer. Journal of Evolutionary Biology 22:1975–1983.

Carvalho, G. R., and R. N. Hughes. 1983. The effect of food availability, female culture-density and photoperiod on ephippia production in Daphnia magna Straus (Crustacea: Cladocera). Freshwater Biology 13:37–46.

Castel, M., L. Mailleret, D. Andrivon, V. Ravigné, and F. M. Hamelin. 2014. Allee Effects and the Evolution of Polymorphism in Cyclic Parthenogens. The American Naturalist 183:E75–E88.

Cavalier-Smith, T. 2002. Origins of the machinery of recombination and sex. Heredity 88:125–141.

Ceh, J., J. Gonzalez, A. S. Pacheco, and J. M. Riascos. 2015. The elusive life-cycle of scyphozoan jellyfish – metagenesis revisited. Nature Publishing Group 1–14.

Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1993. Mutation accumulation in finite outbreeding and inbreeding populations. Genetical Research 61:39–56.

Charlesworth B 1980 The cost of sex in relation to mating system. J Theor Biol 84: 655

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.

Cheptou, P.-O., and F. Massol. 2009. Pollination Fluctuations Drive Evolutionary Syndromes Linking Dispersal and Mating System. The American Naturalist 174:46–55.

Clarke DK, Duarte EA, Elena SF, Moya A, Domingo E, Holland JJ. 1994. The Red Queen reigns in the kingdom of RNA viruses. Proc Natl Acad Sci USA 91: 4821–4824

Clobert, J., M. Baguette, T. G. Benton, and J. M. Bullock. 2012. Dispersal Ecology and Evolution. OUP Oxford.

Cohen, D. 1966. Optimizing reproduction in a randomly varying environment. Journal of Theoretical Biology 12:119–129.

Cohen, D., and S. A. Levin. 1987. The Interaction between Dispersal and Dormancy Strategies in Varying and Heterogeneous Environments. Pages 110–122 inMathematical Topics in Population Biology, Morphogenesis and Neurosciences, Lecture Notes in Biomathematics (Vol. 71). Springer Berlin Heidelberg, Berlin, Heidelberg.

Corley, L.S. and Moore, A.J. 1999 Fitness of alternative modes of reproduction: developmental constraints and the evolutionary maintenance of sex. Proc. R. Soc. Lond. Ser. B 266, 471–476

Cosendai, A.-C., J. Wagner, U. Ladinig, C. Rosche, and E. Hörandl. 2013. Geographical parthenogenesis and population genetic structure in the alpine species Ranunculus kuepferi (Ranunculaceae). Heredity 110:560–569.

Creel, S., Creel, N.M. (1997). Lion density and population structure in the Selous Game Reserve: evaluation of hunting quotas and offtake. *Afr. J. Ecol*. 35, 83:93.

D'Souza, T. G., and N. K. Michiels. 2010. The costs and benefits of occasional sex: theoretical predictions and a case study. Journal of Heredity 101 Suppl 1:S34–41.

Dacks, J., and A. J. Roger. 1999. The First Sexual Lineage and the Relevance of Facultative Sex. Journal of Molecular Evolution 48:779–783.

Dagg, J. L. 2006. Could sex be maintained through harmful males? Oikos 112:232–235.

Darden, S. K., and D. P. Croft. 2008. Male harassment drives females to alter habitat use and leads to segregation of the sexes. Biology Letters 4:449–451.

De Waal, C., B. Anderson, and A. G. Ellis. 2015. Dispersal, dormancy and life-history tradeoffs at the individual, population and species levels in southern African Asteraceae. New Phytologist 210:356–365.

Decaestecker, E., L. De Meester, and J. Mergeay. 2009 Cyclical Parthenogenesis in Daphnia: Sexual Versus Asexual Reproduction. Pages 295–316 *in* I. Schön K. Martens and P. Dijk ed. Lost Sex .

Dodson SI, Frey DG. 2001 Cladocera and other Branchiopoda. In: Thorp A, Covich AP (Eds.) *Ecology and Classification of North American Freshwater Invertebrates, 2nd ed.* pp. 849-913. San Diego, CA: Academic Press.

Doncaster CP, Pound GE, Cox SJ. 2000. The ecological cost of sex. *Nature* 404, 281-285.

Eberhart, A., and K. Tielbörger. 2012. Maternal fecundity does not affect offspring germination - An empirical test of the sibling competition hypothesis. Journal of Arid Environments 76:23–29.

Ebert D, 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books

Ebert, D., Yampolsky, L.Y. 1992. Family planning in *Daphnia*: when is clutch size determined? *Russ. J. Aquat. Ecol*. 1, 143-147.

Edwards, A.W.F. 2000. Carl Düsing (1884) on The Regulation of the Sex Ratio. *Theor. Pop. Biol.* 58, 255-257.

Engelstädter, J., and D. Moradigaravand. 2013. Adaptation through genetic time travel? Fluctuating selection can drive the evolution of bacterial transformation. Proceedings of the Royal Society B: Biological Sciences 281:20132609–20132609.

Engelstädter J. 2008. Constraints on the evolution of asexual reproduction. Bioassays 30: 1138–1150

Evans, M. E. K., and J. J. Dennehy. 2005. Germ banking: Bet-hedging and variable release from egg and seed dormancy. Quarterly Review of Biology 80:431–451.

Fautin, D. G. 2002. Reproduction of Cnidaria. Canadian Journal of Zoology 80:1735–1754.

Fell, P. E. 1989. Porifera. in K. G. Adiyodi and R. G. Adiyodi, eds. Reproductive biology of invertebrates VI. Asexual propagation and reproductive strategies.

Fisher, R.A. 1930. The Genetical Theory of Natural Selection. Clarendon, Oxford, UK.

Fisher, W. K. 1925. Asexual reproduction in the starfish, Sclerasterias. The Biological Bulletin 171–175.

Fitzsimmons JM, Innes DJ. 2006 Inter-genotype variation in reproductive response to crowding among *Daphnia pulex*. *Hydrobiologia* 568, 187-205. (doi: 10.1007/s10750-006-0104-5)

Frank, S.A. 1990. Sex ratio theory for birds and mammals. *Ann. Rev. Ecol. Sys.* 21, 13-55.

Fromont, J., and P. R. Bergquist. 1993. Reproductive biology of three sponge species of the genus Xestospongia (Porifera: Demospongiae: Petrosida) from the Great Barrier Reef

Furness, A. I., K. Lee, and D. N. Reznick. 2015. Adaptation in a variable environment: Phenotypic plasticity and bet-hedging during egg diapause and hatching in an annual killifish. Evolution 69:1461–1475.

Galimov Y, Walser B, and Haag CR. 2011. Frequency and inheritance of non-male producing clones in *Daphnia magna*: evolution towards sex specialization in a cyclical parthenogen? *J. Evol. Biol.* 24, 1572-1583. (doi: 10.1111/j.1420-9101.2011.02288.x)

Gandon, S. 1999. The theory of dispersal under multiple influences. (J. Clobert, T. G. Benton, M. Baguette, & J. M. Bullock, eds.)Journal of Theoretical Biology 200:345–364.

García-Roger, E. M., M. Serra and M. J. Carmona. 2014. Bet-hedging in diapausing egg hatching of temporary rotifer populations - a review of models and new insights. Int. Review Hydrobiol. 99:96-106.

Gerber N, Booksmythe I, Kokko H. *under review.* Bet-hedging and population density shape the timing of facultative sex in unpredictable environments. *Am. Nat.*

Gerber, N., H. Kokko, D. Ebert and I. Booksmythe. *accepted.* Daphnia invest in sexual reproduction when its relative costs are reduced. Proceedings of the Royal Society B

Gibson, A. K., L. F. Delph, and C. M. Lively. 2017. The two-fold cost of sex: Experimental evidence from a natural system. Evolution Letters 1:6–15.

Gilbert J. J.1963. Mictic female production in the rotifer Brachionus calycifloru . J Exp Zool 153: 113–124

Gilbert, J. J., and T. Schröder. 2004. Rotifers from diapausing, fertilized eggs: Unique features and emergence. Limnology and Oceanography 49:1341–1354.

Glesener RR, Tilman D. 1978. Sexuality and the components of environmental uncertainty: clues from geographic parthenogenesis in terrestrial animals. Am Nat 112: 659–673

Goddard M.R., D. Greig, A. Burt. 2001.Outcrossed sex allows a selfish gene to invade yeast populations. Proceedings of the Royal Society B10.1098/rspb.2001.1830.

Goddard KA, Schultz RJ .1993. Aclonal reproduction by polyploid members of the clonal hybrid species Phoxinus eos-neogaeus (Cyprinidae). Copeia 3: 650–660

Gosden, T. P., and E. I. Svensson. 2009. Density-dependent male mating harassment, female resistance, and male mimicry. The American Naturalist 173:709–721.

Gourbière, S., and F. Menu. 2009. Adaptive dynamics of dormancy duration variability: evolutionary trade-off and priority effect lead to suboptimal adaptation. Evolution 63:1879–1892.

Graham, J.K., M. L. Smith and A.M. Simons. 2014. Experimental evolution of bet-hedging under manipulated environmental uncertainty in Neurospora crassa. Proceedings of the Royal society B 281:20140706.

Gravier-Bonnet, N. 1992. Cloning and dispersal by buoyant autotomised hydranths of a Thecate hydroid (Cnidaria; Hydrozoa)

Green, R. F., and D.L.G. Noakes.1995. Is a little bit of sex as good as a lot? Journal of Theoretical Biology, 174, 87–96. http://doi.org/10.1006/jtbi.1995.0081

Gregory, P. H. 1945. The dispersion of air-borne spores. Transactions of the British Mycological Society 28:26–72.

Gregory, P. H. 1961. The Microbiology of the Atmosphere.

Gremer, J. R., E. E. Crone, and P. Lesica. 2012. Are Dormant Plants Hedging Their Bets? Demographic

Gremer, J.R., and D.L. Venable. 2014. Bet-hedging in desert winter annual plants: optimal germination strategies in a variable environment. Ecology Letters 17:380-387.

Gries, T., Jöhnk, K., Fields, D., Strickler, J.R. (1999). Size and structure of 'footprints' produced by *Daphnia*: impact of animal size and density gradients. *J. Plankton Res.* 21, 509-523.

Griffiths, A. M., D. M. P. Jacoby, D. Casane, M. McHugh, D. P. Croft, M. J. Genner, and D. W. Sims. 2012. First analysis of multiple paternity in an oviparous shark, the small-spotted catshark (Scyliorhinus canicula L.). Journal of Heredity 103:166–173.

Griffiths, J. G., and S. P. Bonser. 2013. Is sex advantageous in adverse environments? A test of the abandon-ship hypothesis. The American Naturalist 182:718–725.

Guisande C, Gliwicz ZM. 1992 Egg size and clutch size in two *Daphnia* species grown at different food levels. *J. Plankton Res.* 14, 997-1007.

Gyllström M, Hanson L-A. 2004 Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling. *Aquat. Sci.* 66, 274-295. (doi:10.1007/s00027-004-0712-y)

Hadany, L., and S. P. Otto. 2007. The Evolution of Condition-Dependent Sex in the Face of High Costs. Genetics 176:1713–1727.

Hadany, L., and S. P. Otto. 2009. Condition-Dependent Sex and the Rate of Adaptation. The American Naturalist 174:S71–S78.

Hadfield, M. G. 1975. Hemichordata. Reproduction of Marine Invertebrates V2 185–240.

Hairston Jr. NG, Olds EJ. 1984 Population differences in the timing of diapause: adaptation in a spatially heterogeneous environment. *Oecologia* 61, 42-48. (doi:10.1007/BF00378705)

Halkett F, Harrington R, Hullé M, Kindlmann P, Menu F, Rispe C, Plantegenest M. 2004 Dynamics of production of sexual forms in aphids: theoretical and experimental evidence for adaptive "coin-flipping" plasticity. *Am. Nat.* 163, E112-E125. (doi:10.1086/383618)

Halkett, F., D. Coste, G. G. Rivas Platero, M. F. Zapater, C. AbadieE, and J. Carlier. 2010. Genetic discontinuities and disequilibria in recently established populations of the plant pathogenic fungus Mycosphaerella fijiensis. Molecular Ecology 19:3909–3923.

Halkett, F., Kindlmann, P., Plantegenest, M., Sunnucks, P., Simon, J.C. 2006. Temporal differentiation and spatial coexistence of sexual and facultative asexual lineages of an aphid species at mating sites. *J. Evol. Biol*. 19, 809-815.

Hamilton, W. D. 1963. The evolution of altruistic behavior. American naturalist.

Hamilton WD 1980 Sex versus non-sex versus parasite. Oikos 35: 282–290

Han, C.S., Kang, C-K., Shin, H-S., Lee, J-H., Bae, M-R., Lee, S-I., Jablonski, P.I. 2012. Insects perceive local sex ratio in the absence of tactile or visual sex-specific cues. *Behav. Ecol. Sociobiol.* 66, 1285-1290.

Hanski, I. 1988. Four kinds of extra long diapause in insects: a review of theory and observations. Annales Zoologici Fennici. 25:37-53

Harrison XA. 2014 Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2, e616. (doi:10.7717/peerj.616)

Harrison, P. L., and C. C. Wallace. 2006. Reproduction, dispersal and recruitment of scleractinian corals Ecosystems of the world. 25: Coral Reefs.

Hartfield M, Keightley PD. 2012 Current hypotheses for the evolution of sex and recombination. *Int. Zool.* 7, 192-209. (doi:10.1111/j.1749-4877.2012.00284.x)

Hartfield, M. 2016. Evolutionary genetic consequences of facultative sex and outcrossing. Journal of Evolutionary Biology 29:5–22.

Hebert P.D.N. 1978. The population biology of *Daphnia* (Crustacea, Daphnidae). *Biol. Rev.* 53, 387-426.

Heitman, J. 2010. Sex in Fungi:Molecular Determination and Evolutionary Implications. American Society of Microbiology.

Herre, E.A. (1985). Sex ratio adjustment in fig wasps. *Science* 228, 896-898.

Herre, E.A. (1987). Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature* 329, 627-629.

Hestmark, G. 1992. Sex, size, competition and escape-strategies of reproduction and dispersal in Lasallia pustulata (Umbilicariaceae, Ascomycetes). Oecologia 92:305–312.

Heuschele, J., Selander, E. (2014). The chemical ecology of copepods. *J. Plankton Res.* 36, 895-913.

Hobaek, A., Larsson, P. (1990). Sex determination in *Daphnia magna*. *Ecology* 71, 2255-2268.

Hopper, K. R. 1999. Risk-spreading and bet-hedging in insect population biology. Annu. Rev. Entomol. 44:535–560.

Hörandl, E. 2009. Geographical parthenogenesis: opportunities for asexuality. Pages 161–186 in I. Schön, K. Martens, and P. Dijk, eds. Lost Sex. Springer Netherlands, Dordrecht.

Hurst LD, Peck JR. 1996 Recent advances in understanding of the evolution and maintenance of sex. *Trends Ecol. Evol.* 11, 46-52. (doi:10.1016/0169-5347(96)81041-X)

Hutchinson, J. M. C., and P. M. Waser. 2007. Use, misuse and extensions of "ideal gas" models of animal encounter. Biological Reviews 82:335–359.

Innes DJ. 1997. Sexual reproduction of *Daphnia pulex* in a temporary habitat. *Oecologia* 111, 53-60.

Innes, D. J., C. J. Fox, and G. L. Winsor. 2000. Avoiding the cost of males in obligately asexual Daphnia pulex (Leydig). Proceedings of the Royal Society B: Biological Sciences 267:991–997.

Iritani, R., and P. O. Cheptou. 2017. Joint evolution of differential seed dispersal and self-fertilization. Journal of Evolutionary Biology 30:1526–1543.

Izraylevitch, S., Gerson, U. (1996). Sex allocation by a mite parasitic on insects: local mate competition, host quality and operational sex ratio. *Oecologia* 108, 676-682.

John, B. 1990. Meiosis. Cambridge University Press.

Kahn, A.T., H. Kokko, and M.D. Jennions. 2013. Adaptive sex allocation in anticipation of changes in offspring mating opportunities. Nat. Comm. 4:1603.

Kahn, A.T., Jennions, M.D. & Kokko, H. 2015. Sex allocation, juvenile mortality, and the costs imposed by offspring on parents and siblings. J. Evol. Biol. 28:428-437.

Kawatsu, K. 2013a. Sexually antagonistic coevolution for sexual harassment can act as a barrier to further invasions by parthenogenesis. The American Naturalist 181:223–234.

Kawatsu, K. 2013b. Sexual conflict over the maintenance of sex: effects of sexually antagonistic coevolution for reproductive isolation of parthenogenesis. PloS one 8:e58141.

Kawatsu, K. 2015. Breaking the parthenogenesis fertilization barrier: direct and indirect selection pressures promote male fertilization of parthenogenetic females. Evolutionary Ecology 29:49–61.

Kearney M. 2005 Hybridization, glaciation and geographical parthenogenesis. Trends Ecol. Evol. 20, 495–502.

Kearney, M., and R. Shine. 2005. Lower fecundity in parthenogenetic geckos than sexual relatives in the Australian arid zone. Journal of Evolutionary Biology 18:609–618.

Kleiven OT, Larsson P, Hobaek A. 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65, 197-206.

Klüttgen B, Dülmer U, Engels M, Ratte HT. 1994 ADaM, an artificial freshwater for the culture of zooplankton. *Wat. Res.* 28, 743-746. (doi:10.1016/0043-1354(94)90157-0)

Koch U, von Elert E, Straile D. 2009 Food quality triggers the reproductive mode in the cyclical parthenogen *Daphnia* (Cladocera). *Oecologia* 159, 317-324. (doi:10.1007/s00442-008-1216-6)

Kramer, M.G. and Templeton, A.R. 2001 Life-history changes that accompany the transition from sexual to parthenogenetic reproduction in Drosophila mercatorum. Evolution 55, 748–761

La, G.-H., Choi, J.-Y., Chang, K.-H., Jang, M.-H., Joo, G.-J., Kim, H.-W. (2014). Mating behavior of *Daphnia*: impacts of predation risk, food quantity, and reproductive phase of females. *PLoS ONE* 9, e104545.

Lamb, R.Y. and Willey, R.B. 1979 Are parthenogenetic and related bisexual insects equal in fertility? Evolution 33, 774–775

Lampert, K. P. 2008. Facultative Parthenogenesis in Vertebrates: Reproductive Error or Chance? Sexual Development 2:290–301.

Larsson P. 1991 Intraspecific variability in response to stimuli for male and ephippia formation in *Daphnia pulex*. *Hydrobiologia* 225, 281-290. (doi:10.1007/BF00028406)

Le Galliard, J.-F., Fitze, P.S., Cote, J., Massot, M., Clobert, J. 2005. Female common lizards (*Lacerta vivipara*) do not adjust their sex-biased investment in relation to the adult sex ratio. *J. Evol. Biol.* 18, 1455-1463.

Lee, P. L. M., and G. C. Hays. 2004. Polyandry in a marine turtle: females make the best of a bad job. Proceedings of the National Academy of Sciences of the United States of America 101:6530–6535.

Lehtonen J, Jennions MD, Kokko H. 2012 The many costs of sex. *Trends Ecol. Evol.* 27, 172-178. (doi:10.1016/j.tree.2011.09.016)

Lehtonen J., and H. Kokko 2014. Sex. Current Biology, 24. R305-R306. doi: 10.1016/j.cub.2014.01.060

Lehtonen, J., and H. Kokko. 2012. Positive feedback and alternative stable states in inbreeding, cooperation, sex roles and other evolutionary processes. Philosophical Transactions of the Royal Society B: Biological Sciences 367:211–221.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology & Evolution 183–189.

Levin, S. A., D. Cohen, and A. Hastings. 1984. Dispersal strategies in patchy environments. Theoretical Population Biology 26:165–191.

Lewis W. M. 1987. The cost of sex. In Stearns SC (ed), The evolution of sex and its consequences. Birkhäuser Verlag, Basel, p. 33–57

Li, X.-Y., J. Lehtonen, and H. Kokko. *In press*. Sexual reproduction as bet-hedging. Annals of the International Society of Dynamic Games.

Linkies, A., K. Graeber, C. Knight, and G. Leubner-Metzger. 2010. The evolution of seeds. New Phytologist 186:817–831.

Lively CM. 2010 A review of Red Queen models for the persistence of obligate sexual reproduction. *J. Hered.* 101, S13-S20. (doi:10.1093/jhered/esq010)

Lively CM. 2010. Parasite virulence, host life history, and the costs and benefits of sex. *Ecology* 91, 3-6.

Lively CM. 2011. The cost of males in non-equilibrium populations. *Evol. Ecol. Res.* 13, 105-111.

Lively, C. M., and L. T. Morran. 2014. The ecology of sexual reproduction. Journal of Evolutionary Biology 27:1292–1303.

Lively CM, Jokela J 2002 Temporal and spatial distributions of parasites and sex in a freshwater snail. Evol Ecol Res 4: 219–226

Lockhart, A. B., P. H. Thrall, and J. Antonovics. 1996. Sexually transmitted disease in animals: Ecological and evolutionary implications. Biological Reviews 71:415–471.

Lopez, S., Dominguez, C.A. 2003. Sex choice in plants: facultative adjustment of the sex ratio in the perennial herb *Begonia gracilis*. *J. Evol. Biol*. 16, 1177-1185.

Lynch M. 1983 Ecological genetics of *Daphnia pulex. Evolution* 37, 358-374.

Lynch M. 1989 The life history consequences of resource depression in *Daphnia pulex*. *Ecology* 70, 246-256. (doi:10.2307/1938430)

Lynch, M., and W. Gabriel. 1983. Phenotypic Evolution and Parthenogenesis. The American Naturalist 122:745–764.

Magurran, A. E., and B. H. Seghers. 1994. A Cost of Sexual Harassment in the Guppy, Poecilia reticulata. Proceedings of the Royal Society B: Biological Sciences 258:89–92.

Maldonado, M., and A. Riesgo. 2008. Reproduction in the phylum Porifera: A synoptic overview. Biologia de la reproduccio 1–22.

Martínková, J., and J. Klimešová. 2017. Position of tillers in a clone determines their ontogeny: example of the clonal grass Phalaris arundinacea. Folia Geobot. in press.

Massol, F., and P.-O. Cheptou. 2011. Evolutionary syndromes linking dispersal and mating system: the effect of autocorrelation in pollination conditions. Evolution 65:591–598.

Maynard-Smith, J. 1978. The evolution of sex (Vol. 32). Cambridge University Press.

McAlary, F. A. 1993. Population Structure and Reproduction Iof the Fissiparous Seastar, Linckia Columbiae Gray, on Santa Catalina Island, California. Third California Islands Symposium 233–248.

McCauley E, Nisbet RM, Murdoch WW, de Roos AM, Gurney WSC. 1999. Large-amplitude cycles of *Daphnia* and its algal prey in enriched environments. *Nature* 402, 653-656. (doi:10.1038/45223)

McLain, D.K., Marsh, N.B. 1990. Individual sex ratio adjustment in response to the operational sex ratio in the southern green stinkbug. *Evolution* 44, 1018-1025.

McNamara, J. M., and A. I. Houston. 2009. Integrating function and mechanism. Trends in Ecology & Evolution 24:670–675.

McNamara, J. M., J. N. Webb, E. J. Collins, T. Székely, and A. I. Houston. 1997. A General Technique for Computing Evolutionarily Stable Strategies Based on Errors in Decision-making. Journal of Theoretical Biology 189:211–225.

McNamara, J.M., S.R.X. Dall, P. Hammerstein and O. Leimar. 2016. Detection vs. selection: integration of genetic, epigenetic and environmental cues in fluctuating environments. Ecology Letters. 19:1267-127

Monks, S. P. 1904. Variability and autonomy of phataria. Proceedings of the National Academy of Sciences of Philadelphia 1–7.

Muller, H.1932: Some genetic aspects of sex. American Naturalist 66: 118–138.

Muller HJ 1964 The relation of recombination to mutational advance. Mutat Res 1: 2–9

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed models. *Methods Ecol. Evol.* 4, 133-142.

Nash, T. H. I. 1996. Lichen biology. Cambridge University Press, Cambridge.

Neiman, M. and Koskella, B. et al. 2009 Sex and the Red Queen. in I. Schön, K. Martens, and P. Dijk, eds. Lost Sex. Springer Netherlands, Dordrecht.

Nicolis, S. C., G. Theraulaz, and J. L. Deneubourg. 2005. The effect of aggregates on interaction rate in ant colonies. Animal Behaviour 69:535–540.

Nieuwenhuis, B. P. S., and T. Y. James. 2016. The frequency of sex in fungi. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 371:20150540–12.

Olivieri, I. 2001. The Evolution of Seed Heteromorphism in a Metapopualtion: Interactions Between Dispersal and Dormancy. in J. Silvertown and J. Antonovics, eds. Integrating ecology and evolution in a spatial context.

Olmstead AW, LeBlanc GA. 2001 Temporal and quantitative cycling of the Cladoceran *Daphnia magna* by a juvenile hormone analog. *J. Exp. Zool.* 290, 148-155.

Olsson, M., Shine, R. (2001). Facultative sex allocation in snow skink lizards (*Niveoscincus microlepidotus*). *J. Evol. Biol*. 14, 120-128.

Otto, S. 2008 Sexual Reproduction and the Evolution of Sex. Nature Education 1(1):182

Otto, S. 2009. The Evolutionary Enigma of Sex. *The American Naturalist, 174*(S1), S1-S14. doi:10.1086/599084

Paland, S., Lynch, M. 2006. Transitions to asexuality result in excess amino acid substitutions. *Science* 311, 990-992.

Peck and Waxman. 2000. What's wrong with a little sex? J. Evol. Biol. 13, 63–69

Peck, J. R. 1994. A ruby in the rubbish: beneficial mutations, deleterious mutations and the evolution of sex. Genetics 137:597.

Philippi, T., and J. Seger. 1989. Hedging One's Evolutionary Bets, Revisited. Trends in Ecology & Evolution 1–4.

Pinceel, T., B. Vanschoenwinkel, W. Hawinkel, K. Tuytens, and L. Brendonck. 2017. Aridity promotes bet-hedging via delayed hatching: a case study with two temporary pond crustaceans along a latitudinal gradient. Oecologia 184:161-170.

Pollock, D. A., and B. B. Normark. 2002. The life-cycle of Micromalthus debilisLeConte (1878) (Coleoptera: Archostemata: Micromalthidae): historical review and evolutionary perspective. Journal of Zoological Systematics and Evolutionary Research 40:105–112.

R Core Team. 2016 R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. URL: https://www.R-project.org/

Ram, Y., and L. Hadany. 2016. Condition-dependent sex: who does it, when and why? Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150539–8.

Ramesh, M. A., S.-B. Malik, and J. M. Logsdon. 2005. A Phylogenomic Inventory of Meiotic Genes: Evidence for Sex in Giardia and an Early Eukaryotic Origin of Meiosis. Current Biology 15:185–191.

Rankin, D. J. 2008. Can punishment maintain sex? Oikos 117:173–176.

Rankin D. J., H. Kokko. 2007. Do males matter? The role of males in population dynamics. Oikos 116:335–348.

Ratcliff W. C. and R. F. Denison. 2010. Individual-Level Bet-hedging in the Bacterium Sinorhizobium meliloti. Current biology.https://doi.org/10.1016/j.cub.2010.08.036

Réale, D., P. Boussès, and J.-L. Chapuis. 2011. Female-biased mortality induced by male sexual harassment in a feral sheep population. Canadian Journal of Zoology 74:1812–1818.

Rees, M. 1993. Trade-offs among dispersal strategies in British plants. Nature 366:150–152.

Rice W.R. & Friberg U. 2009: A graphical approach to lineage selection between clonals and sexuals. In Schön I., Martens K. & van Dijk P. (eds): Lost Sex. The Evolutionary Biology of Parthenogenesis. 1st ed. Springer Science + Business Media, Dordrecht, pp. 75–97.

Rice W. R.1987. Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. Genetics 116: 161–167.

Richards A. J. 2003 Apomixis in fl wering plants: an overview. Philos Trans R Soc B Biol Sci 358: 1085–1093

Rieux, A., S. Soubeyrand, F. Bonnot, E. K. Klein, J. E. Ngando, A. Mehl, V. Ravigné, et al. 2014. Long-Distance Wind-Dispersal of Spores in a Fungal Plant Pathogen: Estimation of Anisotropic Dispersal Kernels from an Extensive Field Experiment. (R. A. Wilson, ed.)PloS one 9:e103225–13.

Rispe C, Pierre JS. 1998. Coexistence between cyclical parthenogens, obligate parthenogens, and intermediates in a fluctuatin environment. J Theor Biol 195: 97–110

Rivera, A. C., and J. A. Andrés. 2002. Male coercion and convenience polyandry in a calopterygid damselfly. Journal of Insect Science 2:14.

Robert, K.A., Thompson, M.B., Seebacher, F. (2003). Facultative sex allocation in the viviparous lizard *Eulamprus tympanum*, a species with temperature-dependent sex determination. *Aust. J. Zool.* 51, 367-370.

Rohr, J.R., Park, D., Sullivan, A.M., McKenna, M., Propper, C.R., Madison, D.M. (2005). Operational sex ratio in newts: field responses and characterization of a constituent chemical cue. *Behav. Ecol.* 16, 286-293.

Ronce, O. 2007. How Does It Feel to Be Like a Rolling Stone? Ten Questions About Dispersal Evolution. Annual Review of Ecology, Evolution, and Systematics 38:231–253.

Rönn, J., M. Katvala, and G. Arnqvist. 2006. The costs of mating and egg production in Callosobruchus seed beetles. Animal Behaviour 72:335–342.

Roth, L. M. 1974. Reproductive potential of bisexual Pycnoscelus indicus and clones of its parthenogenetic relative. Annals Of The Entomological Society Of America 215–223.

Roulin AC, Mariadassou M, Hall MD, Walser J-C, Haag CR, Ebert D. 2015 High genetic variation in resting-stage production in a metapopulation: Is there evidence for local adaptation? *Evolution* 69, 2747-2756. (doi:10.1111/evo.12770)

Roulin AC, Routtu J, Hall MD, Janicke T, Colson I, Haag CR, Ebert D. 2013 Local adaptation of sex induction in a facultative sexual crustacean: insights from QTL mapping and natural populations of *Daphnia magna. Mol. Ecol.* 22, 3567-3579. (doi:10.1111/mec.12308)

Rowe, L., G. Arnqvist, A. Sih, and J. J Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends in Ecology & Evolution 9:289–293.

Roze, D., and F. Rousset. 2005. Inbreeding depression and the evolution of dispersal rates: a multilocus model. The American Naturalist 166:708–721.

Ruppert, E. E., R. S. Fox, and R. D. Barnes. 2004. "Lophoporata." Invertebrate Zoology (7 ed.).

Ryland, J. S. 2006. Bryozoa: an introductory overview 1–12.

Scheu, S., and B. Drossel. 2007. Sexual reproduction prevails in a world of structured resources in short supply. Proceedings of the Royal Society B: Biological Sciences 274:1225–1231.

Schierwater B. and C. Hauenschild, 1990. A Photoperiod Determined Life-Cycle in an Oligochaete Worm,*The Biological Bulletin* 178: 111-117.

Schierwater, B., and C. Hauenschild. 1990. A Photoperiod Determined Life-Cycle in an Oligochaete Worm. The Biological Bulletin 178:111–117.

Schmit, O., I. Fukova, J. Vandekerkhove, Y. Michalakis, R. Matzke-Karasz, G. Rossetti, K. Martens, et al. 2013. Mate recognition as a reproductive barrier in sexual and parthenogenetic Eucypris virens (Crustacea, Ostracoda). Animal Behaviour 85:977–985.

Schön, I., K. Martens, and P. Dijk, eds. 2009. Cyclical Parthenogenesis in Daphnia: Sexual Versus Asexual Reproduction. Pages 295–316 inLost Sex. Springer Netherlands, Dordrecht.

Schoustra, S., H. D. Rundle, R. Dali, and R. Kassen. 2010. Fitness-Associated Sexual Reproduction in a Filamentous Fungus. CURBIO 20:1350–1355.

Schröder T, Gilbert JJ. 2004 Transgenerational plasticity for sexual reproduction and diapause in the life-cycle of monogonont rotifers: intraclonal, intraspecific and interspecific variation in the response to crowding. *Funct. Ecol.* 18, 458-466. (doi:10.1111/j.0269-8463.2004.00854.x)

Schröder T. 2005 Diapause in monogonont rotifers. In: Herzig A, Gulati RD, Jersabek CD, May L (Eds.) *Rotifera X: Rotifer Research: Trends, New Tools and Recent Advances, Proceedings of the Xth International Rotifer Symposium*. pp. 291-306. Dordrecht, NL: Springer.

Schurko AM, Neiman M, Logsdon JM Jr (2009) Signs of sex; what we know and how we know it. Trends Ecol Evol 24: 208–217

Schwander, T., and B. P. Oldroyd. 2016. Androgenesis: where males hijack eggs to clone themselves. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150534.

Schwander, T., S. Vuilleumier, J. Dubman, and B. J. Crespi. 2010. Positive feedback in the transition from sexual reproduction to parthenogenesis. Proceedings of the Royal Society B: Biological Sciences 13:rspb20092113–136.

Serra M and King CE. 1999. Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. *J. Evol. Biol.* 12, 263-271. (doi:10.1046/j.1420-9101.1999.00026.x)

Serra M. and Carmona M. J.1993. Mixis strategies and resting egg production of rotifers living in temporally-varying habitats. Hydrobiologia 255–256: 117–126

Serra, M., E. Aparici, and M. J. Carmona. 2008. When to be sexual: sex allocation theory and population density-dependent induction of sex in cyclical parthenogens. Journal of Plankton Research 30:1207–1214.

Serra, M., Snell, T.W. 2009. Sex loss in monogonont rotifers. In: *Lost Sex* (Eds. I. Schön, K. Martens, P. van Dyck), Springer, Dordrecht, NL.

Shine, R., D. O'Connor, and R. T. Mason. 2000. Sexual conflict in the snake den. Behavioral Ecology and Sociobiology 48:392–401.

Simon J-C, Rispe C, Sunnucks P. 2002 Ecology and evolution of sex in aphids. *Trends Ecol. Evol.* 17, 34-39. (doi:10.1016/S0169-5347(01)02331-X)

Slatkin, M. 1974. Hedging One's Evolutionary Bets. Nature 250:704–705.

Smith, C. W., B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James, and P. A. Wolseley. 2009. The lichens of Great Britain and Ireland. British Lichen Society, London.

Snell TW, Garman BL. 1986 Encounter probabilities between male and female rotifers. *J. Exp. Mar. Biol. Ecol.* 97, 221-230. (doi:10.1016/0022-0981(86)90243-1)

Snell TW.1987. Sex, population dynamics and resting egg production in rotifers. Hydrobiologia 144: 105–111

Snyder, R. E. 2006. Multiple risk reduction mechanisms: can dormancy substitute for dispersal? Ecology Letters 9:1106–1114.

Som C, Bagheri HC, Reyer HU. 2007. Mutation accumulation and f tness effects in hybridogenetic populations: a comparison to sexual and asexual systems. BMC Evol Biol 7

Spaak P, Boersma M. 2001 The influence of fish kairomones on the induction and vertical distribution of sexual individuals of the *Daphnia galeata* species complex. *Hydrobiologia* 442, 185-193. (doi:10.1023/A:1017578221814)

Speijer, D., J. Lukeš, and M. Eliáš. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Proceedings of the National Academy of Sciences of the United States of America 112:8827–8834.

Spencer, M. , N. Colegrave, and S. S. Schwartz. 2001, Hatching fraction and timing of resting stage production in seasonal environments: effects of density dependence and uncertain season length. Journal of Evolutionary Biology, 14: 357–367. doi:10.1046/j.1420-9101.2001.00297.x

Sprenger D, Lange R, Anthes N. 2011 Population density and group size effects on reproductive behaviour in a simultaneous hermaphrodite. *BMC Evol. Biol.* 11, 107. (doi:10.1186/1471-2148-11-107)

Starrfelt, J., and H. Kokko. 2012. Bet-hedging-a triple trade-off between means, variances and correlations. Biological Reviews 87:742–755.

Starrfelt, J., and H. Kokko. 2012a. The theory of dispersal under multiple influences

Stelzer C-P and Lehtonen J. 2016 Diapause and maintenance of facultative sexual reproductive strategies. *Phil. Trans. R. Soc. B* 371, 20150536. (doi:10.1098/rstb.2015.0536)

Stelzer C-P. 2012 Population regulation in sexual and asexual rotifers: an eco-evolutionary feedback to population size?. *Funct. Ecol.* 26, 180–188. (doi:10.1111/j.1365-2435.2011.01918.x)

Stelzer C-P. 2016 Extremely short diapause in rotifers and its fitness consequences. *Hydrobiologia* online ahead of issue (doi:10.1007/s10750-016-2937-x)

Stelzer, C. P., and T. W. Snell. 2003. Induction of sexual reproduction in Brachionus plicatilis(Monogononta, Rotifera) by a density-dependent chemical cue. Limnology and Oceanography 48:939–943.

Stelzer, C.-P. 2012, Population regulation in sexual and asexual rotifers: an eco-evolutionary feedback to population size?. Functional Ecology, 26: 180–188. doi:10.1111/j.1365-2435.2011.01918.x

Stelzer, C.-P., and J. Lehtonen. 2016. Diapause and maintenance of facultative sexual reproductive strategies. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150536.

Stelzer, C.-P., and J. Lehtonen. 2016. Diapause and maintenance of facultative sexual reproductive strategies. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150536.

Stross, R.G., Hill, J.C. 1965. Diapause induction in *Daphnia* requires two stimuli. *Science* 150, 1462-1464.

Sugiyama, N., T. Iseto, M. Hirose, and E. Hirose. 2010. Reproduction and population dynamics of the solitary entoproct Loxosomella plakorticola inhabiting a demosponge, Plakortis sp. Marine Ecology Progress Series 415:73–82.

Tarazona, E., E.M. Garcia-Roger, and M.J. Carmona. 2017 *in press*. Experimental evolution of bet-hedging in rotifer diapause traits as a response to environmental unpredictability. Oikos

Taylor BE, Gabriel W. 1993 Optimal adult growth of *Daphnia* in a seasonal environment. *Funct. Ecol.* 7, 513-521. (doi:10.2307/2390126)

Taylor, P. D. 1988. An inclusive Fitness Model for dispersal of Offspring. Journal of Theoretical Biology 363–378.

Tessier, A. J., and C. E. Caceres. 2004. Differentiation in sex investment by clones and populations of Daphnia. Ecology Letters 7:695703.

Tessier, A.J., Goulden, C.E. 1982. Estimating food limitation in cladoceran populations. *Limnol. Oceanogr*. 27, 707-717.

Tielbörger, K., M. Petruů, and C. Lampei. 2012. Bet-hedging germination in annual plants: a sound empirical test of the theoretical foundations. Oikos 121:1860–1868.

Tilquin, A., and H. Kokko. 2016. What does the geography of parthenogenesis teach us about sex? Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150538.

Trail, F. 2007. Fungal cannons: explosive spore discharge in the Ascomycota. FEMS Microbiology Letters 276:12–18.

Trivers, R.L., Willard, D.E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179, 90-92.

Tsuji, N., and N. Yamamura. 1992. A Simple Evolutionary Model of Dormancy and Dispersal in Heterogeneous Patches with Special Reference to Phytophagous Lady Beetles: I. Stable Environments

Umen, J., and J. Heitman. 2013. Evolution of Sex: Mating Rituals of a Pre-Metazoan. CURBIO 23:R1006–R1008.

Van Valen L 1973 A new evolutionary law. Evol Theor 1: 1–30

Venable, D. L., and J. S. Brown. 1988. The Selective Interaction of Dispersal, Dormancy and Seed Size as Adaptations for Reducing Risk in Variable Environments. American naturalist 1–26.

Venable, D. L., C. E. Pake, and A. C. Caprio. 1993. Diversiyt and coexistence of sonoran desert winter annuals. Plant Species Biology 207–216.

Via, S., and R. Lande. 1985. Genotype-Environment Interaction and the Evolution of Phenotypic Plasticity. Evolution 39:505–522.

Vitalis, R., F. Rousset, Y. Kobayashi, I. Olivieri, and S. Gandon. 2013b. The joint evolution of dispersal and dormancy in a metapopulation with local extinctions and kin competition. Evolution 67:1676–1691.

Vrijenhoek 1998 Animal clones and diversity. BioScience. 617-628. doi:10.2307/1313421

Vrijenhoek, R. C., and E. D. Parker. 2009. Geographical Parthenogenesis: General Purpose Genotypes and Frozen Niche Variation. Pages 99–131 in I. Schön, K. Martens, and P. Dijk, eds. Lost Sex. Springer Netherlands, Dordrecht.

Walsh MR. 2013 The link between environmental variation and evolutionary shifts in dormancy in zooplankton. *Integr. Comp. Biol.* 53, 713-722. (doi:10.1093/icb/ict035)

Warner, D.A., Shine, R. (2007). Reproducing lizards modify sex allocation in response to operational sex ratios. *Biol. Lett.* 3, 47-50.

Welnicz, W. W., M. A. Grohme, Ł. Kaczmarek, R. O. Schill, and M. Frohme. 2011. Anhydrobiosis in tardigrades ”The last decade. Journal of Insect Physiology 57:577–583.

Werren, J.H., Charnov, E.L. 1978. Facultative sex ratios and population dynamics. Nature 272, 349-350.

West S.A,. Reece SE, Sheldon BC 2002 Sex ratios. Heredity 88:117–124

West S.A. and Sheldon B.C. 2002 Constraints in the evolution of sex ratio adjustment. Science 295:1685–1688

West, S.A. 2009. *Sex Allocation*. Princeton University Press, Princeton, NJ.

West, S.A., Godfray, H.C.J. 1997. Sex ratio strategies after perturbation of the stable age distribution. *J. Theor. Biol*. 186, 213-221.

Wetherington, J.D. et al. 1987 A test of the spontaneous heterosis hypothesis for unisexual vertebrates. Evolution 41, 721–731

Williams GC. 1975 *Sex and evolution*. Princeton, NJ: Princeton University Press.

Wittmann, M. J., M. A. Lewis, J. D. Young, and N. D. Yan. 2011. Temperature-dependent Allee effects in a stage-structured model for Bythotrephes establishment. Biological Invasions 13:2477–2497.

Worrall, J. J. 2009. Structure and Dynamics of Fungal Populations. (J. J. Worrall, ed.). Kluwer Academic Publishers, London.

Yamaguchi, M., and J. S. Lucas. 1984. Natural parthenogenesis, larval and juvenile development, and geographical distribution of the coral reef asterioid Ophidiaster granifer. Marine Biology 83:33–42.

Yamauchi, A. 1999 Evolution of cyclic sexual reproduction under host–parasite interactions. J. Theor. Biol. 201, 281–291 18.

Yasui, Y. 2001, Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. Ecological Research, 16: 605–616. doi:10.1046/j.1440-1703.2001.00423.x

Zeyl, C. 2009. The role of sex in fungal evolution. Current Opinion in Microbiology 12:592–598.

Zhang L, Baer KN. 2000 The influence of feeding, photoperiod and selected solvents on the reproductive strategies of the water flea, *Daphnia magna*. *Environ. Pollut.* 110, 425-430. (doi:10.1016/S0269-7491(99)00324-3)

Acknowledgments

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I would like to thank Jonna for your help with dealing with everything in Jyväskylä and being part of my thesis and my committee. I would also like to thank all other members of my committee for taking your time for the Skype meetings and supporting me during my PhD.

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A huge thank you goes to the administrative staff for dealing with all the administrative mess with the cotutelle. Special thanks to Anne from the Jyväskylä branch! Without you, I would probably have spent years finding the right things to click on on a Finnish website. Thank you, Petra, for all your support in Zürich. You were a huge help with organizing and booking everything I needed during the whole time! You were not just always there as a secretary, but you also took over duties from Samichlaus and other good fairies and probably some mum-like tasks. Thanks for being the always friendly and positive person in the office!

Thank you, Isobel, for being the best field partner I could imagine. I will never forget the time we spent together in Tvärminne and all of our great plans. You were a great supervisor (not at all a disappointment!) and a great role model. I could learn a lot from you and I admire you as a scientist, but also as a very smart, positive and fun person. I'm not sure how other PhD students manage to finish their PhD without having an Isobel, but I'm sure without you, I would not have been able to complete this thesis as it is now! I would probably be lost somewhere in the archipelago of southern Finland with Alex (or arrested by the Finnish Army…). In addition to your support with the scientific content of this thesis, you were the best co-producer, co-choreographer, motivator, costume designer, etc. for dancing my PhD! Without you, I probably would not have ever realized this great plan! I want to thank you for your support, every discussion and of course for becoming a very dear friend that I really miss having around!

Thank you Anaïs for being a great fellow PhD student, office mate and dear friend! I was very happy when I got a "little sister" in the lab. It turned out that this "little sister" is not just super smart, and also a great inspiration and very helpful for cleaning buckets. It was great to have you there to josh around (does that work?!), but also to talk about serious work stuff. I really enjoyed our meetings where we realized that we envy each other for different traits and I think that I learnt a lot from you. I was definitely enjoying having such a creative mind around, especially when I had some difficulties with writing poems or Haikus! Thank you for

all the hilarious email conversations we had! They were always a welcome distraction and very helpful to procrastinate! I'm sure, wherever I go I will miss hearing the cracking of your feet when you are walking around barefoot!

With Yagmur, we got another PhD sibling! I'm very happy that none of the events I dragged you to scared you off and you are still here! After the lovely film festival and Fasnacht in Solothurn, not even my friend hitting on you at the Pride would stop you from joining all the fun! Thank you for always being so positive, encouraging and sending e-motivation. It was a great pleasure to have you around as a fellow PhD student and good friend. I hope we can still go on that road trip and enjoy lots of other fun events!

As time went on, our Kokkonut Family grew bigger, and with Josh and George, we finally got males in the group who were always up for a chat (or a mumble). Thanks for being part of the Kokkonuts and for every fun and scientific (or both) discussion over lunch, at Friday Pub, GFS, etc. Thanks to every member of the Kokkonuts during the time I was there and also the newest ones for supporting me in the final stages of my PhD! I have spent a lot of time in the past three years in office Y13-H-26. Thanks, Anaïs, Maitane, Sofia, Terhi and all the short-term office mates for making everyday life fun and letting me take a snoring dog to our office!

Thank you Dieter and Jürgen for introducing us to Daphnia ecology, your help and support in Tvärminne, and the very helpful and inspiring discussions in Basel. When I came to Tvärminne the first time, I have never seen Daphnia in the wild, nor have I ever navigated somewhere in the archipelago or driven a boat, let alone mixing ADAM or sexing Daphnia. Without you, this thesis would not have been possible the way it is now! Thank you so much for all your support!

I also want to thank everyone who made my time in Tvärminne so much fun! Thanks for being there for football, frisbee, dancing, cooking, etc. after long days of fieldwork. Special thanks to Charlotte for your help in the field and lab and for being great company in Tvärminne! It was really nice to have you, Siiri, Sanja, and the ants around for so many enjoyable hours on the terrace of the Paviljonki. The same applies to Gutti and the Glowworms, and special thanks to the Pirates for setting the terrace of Paviljonki just a little bit on fire. Not to forget our super awesome "Dance my PhD" team who showed their best dance skills for an amazing production! It was so much fun to do this project with you guys! You are awsome!!! And thanks to everyone from the CoE for the yearly meetings and of course thanks to the CoE for funding and making my PhD even possible. Special thanks to Petri, whom I could bribe into translating my summary! Looking forward to our sword fight was always great motivation to not give up and keep going. I'm looking forward to hearing the clinking of our swords!

A big thank you also to my scientific connection in Bern! Who knows where I would be today without the people in Bern that were preparing me for my PhD far far away in Zürich! Thanks so much to Leif and Arne for the long hours with Otto and Day and for accompanying me in my very first steps in modeling. But who knows where I would be today if I would not have had this beer with Jo back then in Arolla, where you encouraged me to approach Hanna Kokko. What probably also helped with this important step was my generally increased confidence through all the Töggeli-victories against you in the past which optimally prepared me for this PhD! Dear Manon, thanks for shaping my early scientific career during my Bachelor

and Master studies and for being a role model for me to look up to. Also, you always took the time to discuss the overdue manuscript, which won't be published before I finish my PhD…

Thank you, Maya and Nice and many others for your support during my studies and during my PhD. It was always nice and refreshing to have people around who supported me outside of academia. Thanks to the whole Rugby Club Bern for letting me sometimes forget that I should actually work on my thesis instead of getting bruised on the pitch. Also thanks for never breaking my nose or giving me a concussion so that I forget the title of my thesis!

My early interests in nature and biology were reinforced by growing up in a garden with huge biodiversity and parents that appreciated and admired this diversity. I would like to thank my mum and dad for teaching me to be curious about nature and for letting me explore it as much as I wanted! Also, I should thank you (and probably apologize) for the many times you had to get up at 5 am to go to explore the beach during holidays, or to go to the forest to watch deer and foxes jumping around on weekends. At this point, I should probably also thank my brother for forming my personality! After being shot by your brother with a dart, there is not much out there that impresses you… so thank you for giving me this experience early in life so that I could take this PhD with ease.

Especially I want to thank you, Anouk, for supporting me within and outside of academia. Thank you so much for being the best support for writing this thesis! I am so grateful for your patient efforts in trying, to teach me, where commas and dashes belong to and where they – don't. Thank you for always believing in me and for convincing me that I'm not actually stupid because I did something pretty stupid… I am not only grateful for your help with my thesis, but also for your unconditional love and support in my life in general. I cannot write enough to express how much I appreciate having you around. But I can say that I am pretty happy about the fact that sex is maintained, because as Hamilton said, otherwise we might no longer have love.

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CV and Research Output

Personal information

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Education

03.2015 – present	**PhD candidate**, Department for Evolutionary Biology and Environmental Studies, University of Zurich, and Department of Biological and Environmental Science, University of Jyväskylä, Finland, supervised by Prof. Dr. Hanna Kokko Expected date of PhD defence: 02.03.2018
09.2013 – 02.2015	**MSc in Ecology and Evolution** with special qualification in Behavioural Ecology, University of Bern (summa cum laude); Master thesis: „Olfactory communication in a cooperative dilemma in Norway rats“, supervised by PhD candidate Manon Schweinfurth and Prof. Dr. Michael Taborsky
09.2010 – 12.2013	**BSc Biology** with special qualification in Ecology and Evolution, University of Bern; Bachelor thesis: “No direct reciprocal cooperation in the absence of olfactory information in female Norway rats (*Rattus norvegicus*)”, supervised by Prof. Dr. Michael Taborsky

Employment history

03.2015 – present	**Research assistant as PhD candidate**, Department for Evolutionary Biology and Environmental Studies, University of Zurich, and Department of Biological and Environmental Science, University of Jyväskylä, Finland, advised by Prof. Dr. Hanna Kokko

05.2014 – 09.2014 **Field assistant** to collect and identify plants and visiting pollinators in different field sites in the Bernese pre-alps, Switzerland Community Ecology, Institute of Ecology and Evolution, University of Bern, advised by Dr. Eva Knop,

Approved research projects

2013 License to perform animal experiments for the project

"Proximate Mechanisms of Reciprocal Cooperation in Norway Rats"

Main applicant: Prof Dr. Michael Taborsky

Co-applicants: Manon Schweinfurth, Nina Gerber

Teaching activities

Training

10.2015/01.2016 First steps into science teaching and learning in Biology, University of Zurich

Teaching

Spring semesters 2016/ 2017 Lecturer in exploitation of renewable resources, University of Zurich; Topics:

-Spatial conservation and marine protected areas

-Biofuels and indirect land use change

-Sustainable harvesting in age-structured populations

Spring semester 2017 Teaching assistant in a practical course on the statistical analysis of biological data , University of Zurich

Spring semester 2016 Teaching of a MatLab practical course on sustainable harvesting in age structured populations, University of Zurich

Fall semester 2016 Teaching assistant in a practical course on classical and molecular genetics , University of Zurich

Membership in panels, boards, etc., and individual scientific reviewing activities

2016 Reviewer for Philosophical Transactions of the Royal Society B

Active memberships in scientific societies, fellowships in renowned academies

British Ecological Society, Swiss Zoological Society, Eroupean Society for Evolutionary Biology

Organisation of conferences

06.2017 Organistaion of a PhD retreat in Evolutionary Biology, Moeschberg, Switzerland

Prizes, awards, fellowships

2016	Best talk award at Biology16
2015	Best talk award (Runner up) at Biology15

Personal skills

Language skills

German	Native
English	Fluent
French	Basics

Digital competences

Data and literature management	Literature search databases and citation tools (e.g. Papers, Endnote, Mendeley), Data Dryad
Programming languages / Computation	MatLab, R, LateX, Mathematica
Illustration	Photoshop, Adobe Illustator, Premiere CC Pro

Additional scientific training

2017	Niche modelling, 2 days introductory course at the University of Basel
2016	Introduction to evolutionary and population ecological modeling, 1 week course at the University of Zurich
	MatLab for biologists, 2 days course at ETH Zurich

2015	Introduction to computational biology, 2 days course at the University of Zurich
2013	Field course in marine biology: Planning and conducting a 4-week project on the cooperative dilemma in cleaner fish with their host species at the HYDRA Institute for Marine Biology, Elba.
	Field course in conservation biology: Planning and conducting a 4 week project on habitat preferences of threatened Orthopterans, Valais

Publications

Published

Fischer, S., E. Oberhummer, F. Saraiva, **N. Gerber** & B. Taborsky, **2017**. Smell or vision? The use of different sensory modalities in predator discrimination. *Behavioral Ecology and Sociobiology,* https://doi.org/10.1007/s00265-017-2371-8

Gerber, N., & H., Kokko, **2016**. Sexual conflict and the evolution of asexuality at low population densities. *Proceedings: Biological Sciences*, *283*(1841), 20161280 http://doi.org/10.1098/rspb.2016.1280

Gerber N., H. Kokko, D. Ebert, & I. Booksmythe, *accepted.* Daphnia invest in sexual reproduction when its relative costs are reduced. *Proceedings Biological Science*

Submitted but not yet accepted

Gerber, N., M.K. Schweinfurth, & M. Taborsky, *under review*. The smell of cooperation: Rats reciprocate help solely based on odour cues. *Science*

Gerber N., I. Booksmythe, & H. Kokko, *under review.* Bet-hedging and population density shape the timing of facultative sex in unpredictable environments. *American Naturalist*

Booksmythe I., **N. Gerber**, D. Ebert, & H. Kokko, *under review*. Daphnia females adjust sex allocation in response to current sex ratio and density. *Ecology Letters*

In preparation

Gerber N. & H. Kokko, *in preparation*. The coevolution of sexual reproduction and dispersal in facultatively sexual organisms.

Contributions to international conferences

2017 Oral presentation at the meeting of the European Society of Evolutionary Biology (ESEB), Groningen, Netherlands: *The coevolution of sex and dispersal*

2016 Oral presentation at Biology16, Lausanne, Switzerland: *Male sexual harassment and the evolution of asexuality at low population densities*

Oral presentation at the annual meeting of the British Ecological Society (BES), Liverpool, UK: *The coevolution of sex and dispersal and the evolution of dispersal through kin competition*

2015 Oral presentation at the European Meeting of PhD Students in Evolutionary Biology (EMPSEB) 21, Stirling, UK: *When to have sex? A big question in the life of cyclical partheongens*

Oral presentation at Biology15, Dübendorf, Switzerland: *The smell of cooperation: Norway rats reciprocate help based on odour cues alone*

2014 Oral Presentation at the European Conference on Behavioural Biology (ECBB), Prague, Czech: *Norway rats need olfactory information to cooperate reciprocally in an iterated prisoner's dilemma game*

Outreach activities

2017 Scientific Speed Dating at Biology17, University of Bern

2016 Sientific contribution to an exhibition at the Zoological Museum Zurich:

- Developing a comic to the topic "Fakultativer Sex, das Beste zweier Welten" / "Facultative sex, the best of both worlds", in collaboration with Tara Gschwend (artist) from Life Science Zurich.

- Guided tour at the Zoological Museum of Zurich: "Sex aus der Sicht einer Biologin" / "Sex from a biologist's point of view"

-Guided tour at the Zoological Museum of Zurich: "Fakultativer Sex, das Beste zweier Welten" / "Facultative Sex, the best of both worlds"

Sientific outreach activity at Biology16 including poster exhibition, University of Lausanne

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